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CYTOGENETIC STUDIES ON *TRITICUM AESTIVUM* × *AEGILOPS CYLINDRICA* HYBRIDS AND DERIVATIVES

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Developing chromosome addition lines is one of the promising ways of transferring useful genes from wild into cultivated species, via homoeologous recombination. *Aegilops cylindrica* is one of the *Aegilops* species that have gametocidal gene(s), causing chromosomal rearrangements. In this paper the development and cytological characterization of some *Triticum aestivum/Ae. cylindrica* addition lines are described.

Key words: *Aegilops cylindrica*, addition lines, Giemsa C-banding, *Triticum aestivum*

Introduction

Aegilops cylindrica Host (2n = 4x = 28; genomically $C^cC^cD^cD^c$) is one of the intensively investigated wild relatives of cultivated bread wheat (*Triticum aestivum* L. em. Thell., 2n = 6x = 42; genomically AABBDD - taxonomic nomenclature after van Slageren, 1994) for two reasons. One of them is practical: this species is an important source for broadening the genetic variability of cultivated wheat with agronomically useful resistance genes, i.e. resistance to many diseases, such as leaf and stem rust (Bai et al., 1995) and snow mould (Iriki, 1994), and tolerance to different abiotic stresses such as salt tolerance (Farooq et al., 1995) and frost resistance (Sutka, 1994). On the other hand *Ae. cylindrica* has theoretical importance, too, because it contains gametocidal genes causing chromosomal rearrangements (Endo, 1982; 1988a; 1988b) and chromosome deletions (Endo and Gill, 1996) when transferred into common wheat or into other crop species. This system has been used to establish a set of more than 400 deletion stocks in Chinese Spring wheat (Endo and Gill, 1996). It is possible to map physically different genes with the help of these deletion lines (Endo and Gill, 1996).

There are other *Aegilops* species e.g. *Ae. speltoides* Tausch (SS, 2n = 2x = 14), *Ae. triuncialis* L. ($U^tU^tC^tC^t$, 2n = 4x = 28), *Ae. sharonensis* Eig (S^sS^s, 2n = 2x = 14) and *Ae. longissima* Schweinf. & Muschl. (S^l, 2n = 2x = 14) also possessing gametocidal genes (Endo, 1990).

Chromosome banding techniques (C-banding, N-banding) make it possible to identify chromosomes in many plant species and in their hybrids and

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amphiploids (Gill and Kimber, 1974a; Gill and Kimber, 1974b; Endo and Gill, 1984). Giemsa C-banding allows the identification of all 21 pairs of chromosomes in hexaploid wheat (Gill et al., 1991) and in addition it is widely used for chromosome identification in many other species relative to wheat such as *Aegilops caudata* L. (CC, 2n = 2x = 14) (Friebe et al., 1992a) and *Aegilops tauschii* Coss. (DD, 2n = 2x = 14) (Friebe et al., 1992b).

Developing chromosome substitution and/or addition lines is one of the promising ways of transferring useful genes from wild into cultivated species, via homoeologous recombination and translocation (Jiang et al., 1994; Friebe et al., 1996).

In the present work results achieved in the development and cytological examination of *Triticum aestivum/Ae. cylindrica* addition lines are described.

Materials and methods

The material used in this experiment was four accessions of *Aegilops cylindrica* Host (provided by A. Belea; Belea713, Belea2018, Belea 10-259, Belea 10-279 and the *Triticum aestivum* L. varieties Chinese Spring and Martonvásári 14. The development of disomic additions was carried out according to the scheme presented in Figure 1. Ten ears from each combination were crossed.

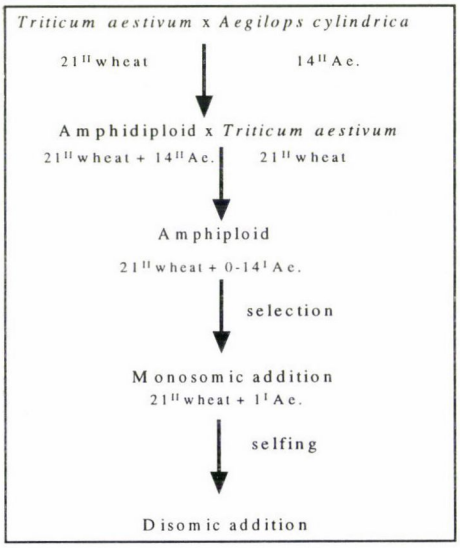

Fig. 1. Scheme for developing *Triticum aestivum* × *Aegilops cylindrica* disomic addition lines

For chromosome identification the standard C-banding technique described by Gill et al. (1991) was used. The homeology of the *Ae. cylindrica* chromosomes was determined by comparison with the C-banded karyotypes of the progenitor species *Aegilops tauschii* (Friebe et al., 1992b) and *Aegilops caudata* (Friebe et al., 1992a).

Results

The meiotic pairing of *T. aestivum* × *Ae. cylindrica* and *Ae. cylindrica* × *T. aestivum* F_1 hybrids is summarized in Table 1.

Table 1.
Meiotic pairing of *T. aestivum* × *Ae. cylindrica* and *Ae. cylindrica* × *T. aestivum* F_1 hybrids

F_1 combination	Chr. No. in root tip	No. of meiotic cells examined	Chromosome associations						
			Uni-valent	Bivalent			Tri-valent	Quadri-valent	Fragment
				Ring	Rod	Total			
CS× *Ae. cyl.*	35	153	23.7	2.4	2.4	4.8	0.30	0.01	0.3
Mv14 × *Ae. cyl.*	35	62	23.3	2.9	2.4	5.3	0.20	0.04	0.3
Ae. cyl. × CS	35	92	19.5	3.9	3.0	6.9	0.05	0.00	0.3
Ae. cyl. × Mv14	35	274	19.5	3.8	3.1	6.9	0.20	0.01	0.1

The mean chromosome number is 33.3 (23.7 + 2 · 4.8) in the CS × *Ae. cylindrica* combination, and 33.9 (23.3 + 2 · 5.3) in the Martonvásári 14 × *Ae. cylindrica* combination, respectively (Table 1). In the case of both *Ae. cyl.* × Chinese Spring and *Ae. cyl.* × Mv14 the mean value of the chromosome number was 33.3 (19.5 + 2 · 6.9). Taking into account the trivalents this number is close to the theoretically expected 35. But the mean number of bivalents depends on the direction of crossing and is higher if *Ae. cylindrica* is the maternal parent (Belea, 1986).

The typical meotic pairing (7^{II} and 21^{I}) is shown in Figure 2, indicating that *T. aestivum* and *Ae. cylindrica* have one genome (D) in common.

Fig. 2. Meiotic pairing of a *T. aestivum* × *Ae. cylindrica* F_1 hybrid (7^{II} and 21^{I})

C-banding pattern of Ae. cylindrica

The C-banding patterns of the D^C-genome chromosomes of *Ae. cylindrica* are very similar to those of the D-genome progenitor species *Ae. tauschii* (Fig. 3). This similarity allows us to assign all D^C-genome chromosomes of *Ae. cylindrica* to their homoeologous groups. The C-banding patterns of the C^C-genome chromosomes of *Ae. cylindrica* are also similar to the corresponding chromosomes of the C-genome progenitor species *Ae. caudata*. So far only three chromosomes of *Ae. caudata* have been assigned to their homoeologous groups. Chromosome 1C (designated as A by Friebe et al., 1992a) is missing in the set of *T. aestivum*/*Ae. caudata* addition lines developed by Blüthner and co-workers (Blüthner et al., 1988) but is available in the form of a 1C (1D) chromosome substitution line developed by Kihara and described by Muramatsu (1959). Chromosome 5C compensates for the loss of chromosome 5D in a derived substitution line, indicating that this chromosome belongs to group 5. This chromosome was designated as chromosome C by Friebe et al. (1992a). The gametocidal chromosome of *Ae. cylindrica* transferred by Endo and used to produce a set of chromosome deletions in common wheat (Endo and Gill, 1996) is substituted for group 2 chromosomes in derived substitution lines, indicating its homoeology to the group 2 chromosomes of wheat. This chromosome is similar in morphology and C-banding pattern to the *Ae. caudata* designated as B

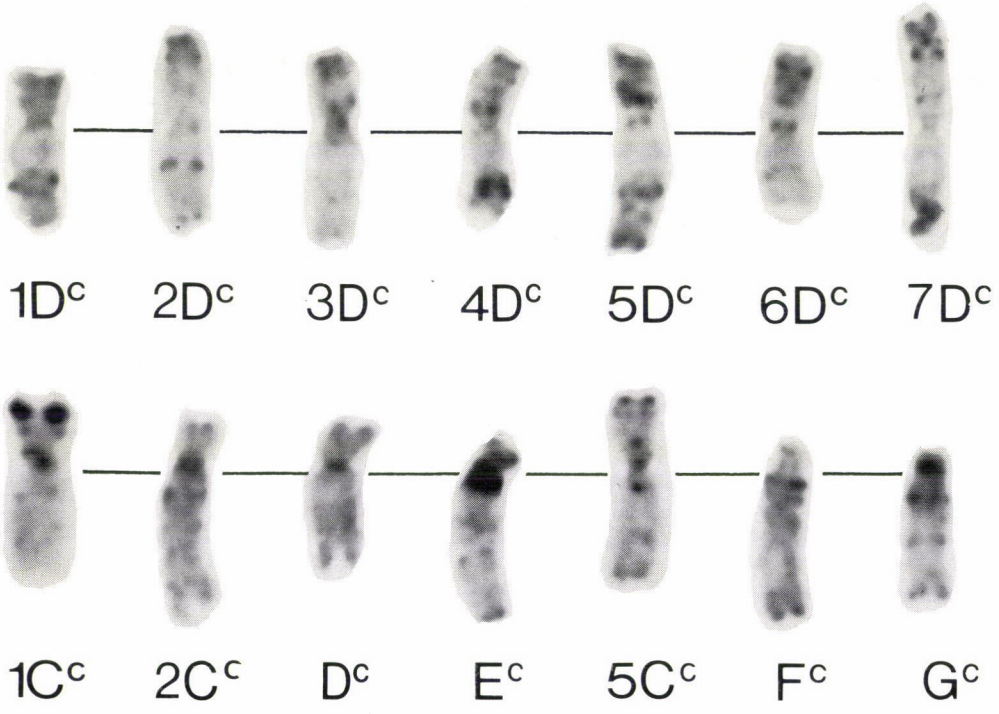

Fig. 3. C-banded karyotype of *Aegilops cylindrica*

in the set of addition lines described by Friebe et al. (1992a). The remaining four C-genome chromosomes of *Ae. caudata* showed homoeology to more than one group according to isozyme (Schmidt et al., 1993) and RFLP analysis (K.S. Gill, personal communication). Because the homoeology of these chromosomes needs further elaboration, they were designated as D, E, F and G according to their similarity in C-banding patterns and morphologies with the corresponding *Ae. caudata* chromosomes (Friebe et al., 1992a).

Whereas C-band polymorphism was observed in *Ae. tauschii* as well as in *Ae. caudata* only minor variation in C-band size and C-band position is present in the D and C genomes of *Ae. cylindrica* (unpublished data).

Because of the high degree of sterility most of the combinations were lost during the crossing. The distribution of chromosome number after the second backcross is presented in Table 2.

Table 2
Chromosome number distribution after the second backcross in offsprings

Combination	48	47	46	45	44	43	42	41	Unknown	Total
CS × *Ae. cyl.*	–	–	2	2	22	8	23	–	14	71
Mv14 × *Ae. cyl.*	4	–	–	–	2	1	–	–	3	10

The chromosomal constitution of lines with 2n=44 and 46 chromosomes was determined by C-banding. Figure 4 shows the *Ae. cylindrica* and two of the rearranged (abnormal) wheat chromosomes found in these lines. The presence of dicentrics and deletions indicate that this *Ae. cylindrica* accession contains a gametocidal gene on $2C^C$ chromosome. In most of the 44 chromosome lines this chromosome is present, confirming its gametocidal effect because of the preferential transmission of the gametocidal chromosome. Therefore, the *cylindrica* accessions used in the present work are not suitable for the production of all the disomic additions.

Chromosome pairing analysis in the F_1 hybrid *T. aestivum* × *Ae. cylindrica* showed that the D and D^C genomes form chromosome associations (max. 6^{II} ring and 1^{II} rod) at meiotic metaphase I. Thus, the D genome chromosomes in the derived chromosome addition line should be highly recombined and derived from both the D genome of *T. aestivum* and the D^C genome of *Ae. cylindrica*. In order to extract the pure D^C genome out of *Ae. cylindrica* either crosses between the appropriate D-genome Chinese Spring monosomics and *Ae. cylindrica* should be made, which will result in the establishment of a set of D^c genome substitution lines into Chinese Spring, or the initial F_1 hybrid should be made with *T. turgidum,* i.e. the extracted tetraploid Chinese Spring line produced by Kerber (1964).

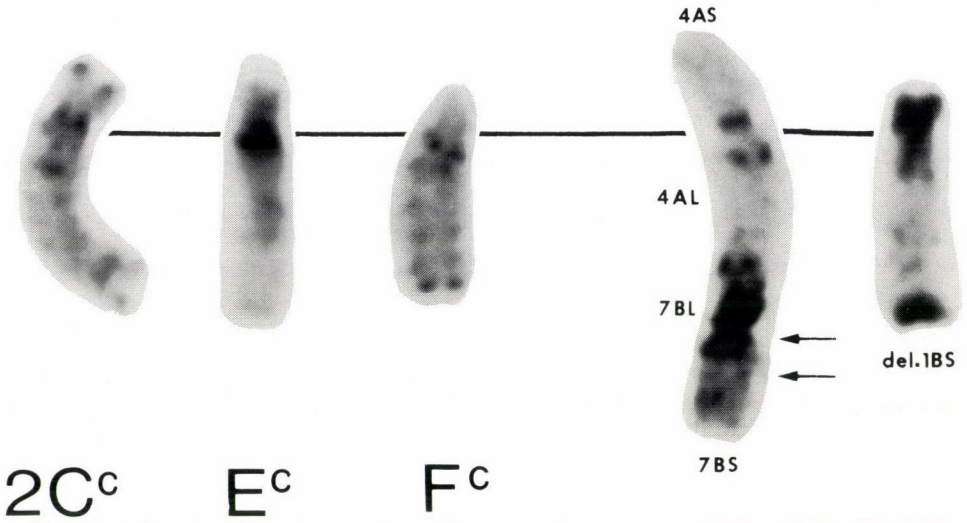

Fig. 4. C-banding pattern of the *Aegilops cylindrica* and the abnormal wheat chromosomes found in BC_2 progeny

Acknowledgements

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STANDARDIZATION OF THE BEST TIMING OF GROWTH REGULATOR APPLICATION IN *GOSSYPIUM HIRSUTUM* AND *G. ARBOREUM* CROSSES TO INCREASE BOLL FORMATION AND SEED SET

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The effect of gibberellic acid (GA_3) and indole acetic acid (IAA) on boll formation and seed set was studied after applying these growth regulators to the flowers of *Gossypium hirsutum* and *G. arboreum* crosses before and after pollination in different seasons. The maximum hybrid boll formation was recorded in the kharif season in 'Vikas' × 'Lohit' (36.3%) when 100 ppm GA_3 was applied, but a larger number of hybrid seeds/boll was obtained in the reciprocal when 100 ppm IAA was applied. The best time for growth regulator application was at pollination in the kharif season, when 40.4% boll formation was recorded.

Key words: *Gossypium hirsutum, G. arboreum,* growth regulator, GA_3, IAA

Introduction

Pollination appears to induce increased auxin levels in the pistils and, for angiosperms at least, after fertilization the developing seeds seem to be the primary sites of auxin synthesis in the developing fruits. Pollen extracts and plant growth substances can be used to stimulate the growth of the ovary in the absence of pollination (Holloway et al., 1982; Saavedra, 1979). It is thus probable that the initial set of the fruit is triggered by growth substances liberated into the pistil tissue from the pollen. The quantities of growth regulators released by the pollen, however, seem inadequate to account for continued fruit growth. Attempts have been made to overcome this problem with the judicious application of naturally occurring and synthetic plant growth substances by many investigators (Wang and Chen, 1984; Altman, 1988; Kodali and Khanna, 1994). This study was undertaken to standardize the most suitable growth regulator concentrations and their time of application to the flowers in order to obtain maximum boll set and seed set in interspecific crosses of cotton.

Materials and methods

The experimental material used in the present study comprised one cultivar each of tetraploid cotton *(Gossypium hirsutum)* and diploid cotton *(G. arboreum)*, namely 'Vikas' and 'Lohit', respectively. The seeds were surface-sterilized for 10 minutes in 0.2% mercuric chloride.

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They were washed in distilled water and sown in the field using a randomized block design with the split–split plot technique, once in spring (01.03.1994) and once in the Kharif season (18.06.1994). Twenty plants, each with 10 flowers, were used per treatment. The following crosses were made:

1. 'Vikas' × 'Vikas'
2. 'Lohit' × 'Lohit'
3. 'Vikas' × 'Lohit'
4. 'Lohit' × 'Vikas'

In the spring-sown crop the crossing was done in May–June and in kharif-sown crops the crossing was done during September–October.

The emasculation of the flowers was carried out in the afternoon, taking care not to injure the delicate gynoecium and bagging was done by inserting soda straw over the gynoecium. Pollination was carried out the next morning between 7–10 A.M. Two different growth regulators, namely gibberellic acid (GA_3) and indole acetic acid (IAA) at concentrations of 50 and 100 ppm each, applied separately or in combinations of GA_3 (50 ppm) + IAA (100 ppm) and GA_3 (100 ppm) + IAA (50 ppm), were applied to the base of the gynoecium at emasculation, at pollination and 48 hours after pollination. Pollinated boll samples were taken at maturity to calculate the percentage boll formation and the seed set per boll. Data analysis was done using a randomized block design with the split–split plot technique. The percentage data were analysed after angular transformation.

Results

Significant differences in the number of bolls formed, in boll growth and in seeds/bolls were observed between crosses, growth regulators and the times of growth regulator application (Table 1).

In spring a greater number of bolls was obtained in the case of selfing (49.5%) than in the crosses (18.7%), while in the crosses 'Lohit' × 'Vikas' gave more bolls than the reciprocal cross except in the case of IAA (50 ppm), where the trend was reversed (Table 2).

Table 1

Analysis of variance (Mean square) for different quantitative characters in cotton

Source	df	% boll formation		Boll growth		Seeds/boll	
		Spring	Kharif	Spring	Kharif	Spring	Kharif
Cross (C)	3	5811.8**	6911.3**	17.5**	37.1**	2910.7**	6792.4**
Error (a)	3	0.4	3.0	0.04	0.3	5.6	1.9
Growth regulator (G)	6	266.2**	258.4**	0.2**	0.2**	36.9**	22.2**
C × G	18	41.0	108.1**	0.16**	0.2	28.5**	14.4**
Error (b)	24	2.8	3.6	0.04	0.2	8.4	3.2
Time$^+$ (T)	2	417.1**	80.9**	0.1	0.3	28.3**	47.2
C × T	6	107.9**	160.0**	0.3	0.3	91.8**	41.8**
G × T	12	90.1**	100.6**	0.2	0.2	12.3**	13.8**
C × G × T	36	35.4**	36.1**	0.1	0.2**	13.2**	16.8**
Error (C)	56	1.2	1.3	0.2	0.05	2.9	2.5

$^+$ Time of growth regulator application, ** = Significant at 1% level, C = Cross, G = Growth regulator, T= Time of growth regulator application

Table 2
Percentage boll formation in different seasons and different growth regulator concentrations (H1 – H7) in cotton

Season	Cross	Hormones							
		H1	H2	H3	H4	H5	H6	H7	Mean
Spring	'Vikas' × 'Vikas'	47.5	57.1	66.7	56.3	61.7	46.3	48.3	54.8
	'Lohit' × 'Lohit'	28.8	49.2	55.0	39.6	47.1	43.8	45.4	44.1
	'Vikas' × 'Lohit'	11.3	17.1	21.3	16.3	14.6	17.5	20.8	17.0
	'Lohit' × 'Vikas'	17.8	22.9	25.8	14.1	19.2	18.8	24.1	20.4
	Mean	26.3	36.6	42.2	31.5	35.6	31.6	34.7	34.1
Kharif	'Vikas' × 'Vikas'	53.8	74.6	75.0	76.3	68.8	54.2	58.4	65.8
	'Lohit' × 'Lohit'	53.8	44.2	53.8	45.4	54.6	48.3	39.8	48.5
	'Vikas' × 'Lohit'	12.5	29.2	36.3	17.1	22.1	25.0	30.3	24.6
	'Lohit' × 'Vikas'	15.8	25.4	33.8	19.2	17.9	19.2	22.6	22.0
	Mean	33,9	43.3	49.7	39.5	40.8	36.6	37.8	40.2

H1 = Control; H2 = GA_3 (50 ppm); H3 = GA_3 (100 ppm); H4 = IAA (50 ppm); H5 = IAA (100 ppm); H6 = GA_3 (50 ppm) + IAA (100 ppm); H7 = GA_3 (100 ppm) + IAA (50 ppm)

The best growth regulator was 100 ppm GA_3 with a 42.2% rate of boll formation (Table 2). The best time for growth regulator application was found to be at the time of pollination (Table 3).

In the kharif season the maximum number of bolls was obtained after selfing, as in spring. The greatest boll formation in the case of the crosses was seen in 'Vikas' × 'Lohit' (24.6%), whereas the reciprocal cross gave a value of 22%. The best growth regulator was found to be 100 ppm GA_3 (49.7%, Table 2), while the best time for growth regulator application was at the time of pollination (Table 3).

Table 3
Percentage boll formation at different times of growth regulator application in different seasons

Time	Season	Cross				Mean
		V × V	L × L	V × L	L × V	
Emasc.	Spring	54.6	40.5	15.7	20.5	32.8
	Kharif	60.7	43.3	26.1	23.5	38.4
	Mean	57.7	41.9	20.9	22,0	35.7
Pollinat.	Spring	64.5	45.0	20.0	24.6	38.5
	Kharif	67.1	48.2	25.7	27.6	42.2
	Mean	65.8	46.6	22.9	26.1	40.4
48 hours after pollinat.	Spring	45.4	46.8	15.2	15.2	30.7
	Karif	69.7	54.0	22.1	14.8	40.2
	Mean	57.6	50.4	18.7	14.6	35.3

V = Vikas, L = Lohit

The growth of crossed bolls was very slow as compared to that of selfed bolls in both the spring and kharif seasons. Selfed bolls reached a maximum length of 3.5 cm in the case of 'Vikas' when 100 ppm GA_3 was applied in spring. Bolls of the same variety attained a length of 4.1 cm when the growth regulator combination GA_3 (100 ppm) + IAA (50 ppm) was applied in the kharif season. Maximum hybrid boll lengths of 2.1 cm and 2.4 cm were obtained for 'Vikas' × 'Lohit' in spring and kharif, respectively, when IAA (50 ppm) was sprayed. The boll length was higher in kharif than in spring in all cases.

When the hybrid bolls were examined for seeds, very small, shrunken seeds were seen. Normal, healthy seeds were seen in the case of selfing. The number of seeds/boll was very high after selfing as compared to the crossed bolls (Table 4). A greater number of seeds was found in kharif than in spring after both selfing and crossing. In spring the best growth regulator for seeds/boll in 'Vikas' × 'Lohit' was 100 ppm GA_3 with 0.5 seeds per boll, while for 'Lohit' × 'Vikas' it was the growth regulator combination GA_3 (50 ppm) + IAA (100 ppm) with 5.2 seeds/boll. In kharif the best growth regulator was found to be GA_3 (100 ppm) for 'Vikas' × 'Lohit' with 0.3 seeds/boll and IAA (100 ppm) for 'Lohit' × 'Vikas' (10.8 seeds/boll).

Discussion

Early boll abscission was found to be the main post-fertilization barrier and this eventually decreased the percentage boll harvest considerably in crosses between *Gossypium* species. The percentage boll formation ranged from 28.8–66.7% in the parents and from 11.3–25.8% in the crosses (Table 2) in spring. In kharif the range was 39.8–76.3% and 12.5–36.3% in parents and crosses, respectively. Boll formation was higher in kharif than in spring.

Growth regulator application increased the boll harvest in both the parents and the crosses. The proportionate increase in boll formation due to growth regulator application was greater in the crosses than in the parents. The most effective growth regulator for boll formation was found to be GA_3 at a concentration of 100 ppm (Table 2). The positive effect of GA_3 on crosses of *Gossypium* was reported by Sandhu and Brar (1983) and Altman (1988). This may be due to better germination and pollen tube growth. GA_3-induced pollen tube elongation is considered to be due to the effect of cell expansion and the orientation of newly-synthesized microfibrils.

Boll length was greater (3.1 cm) in the parents than in the crosses (2.0 cm) and greater in the kharif season than in spring. Growth regulator treatments showed a wide range of effects on the growth (Table 4). A maximum boll length of 4.1 cm was observed in the parent 'Vikas' when the growth regulator combination GA_3 (100 ppm) + IAA (50 ppm) was applied in the kharif season, whereas in the crosses a maximum boll length of 2.4 cm was recorded in the

Table 4

Effect of growth regulator concentrations (H1–H7) and seasons on boll length (cm) and seeds per boll in selfed and crossed bolls of cotton

Season	Growth regulator	Boll length (cm)						Seeds/boll					
		V×V	L× L	Mean (Selfed)	V×L	L×V	Mean (Crossed)	V×V	L ×L	Mean (Selfed)	V×L	L ×V	Mean (Crossed)
Spring	H1	3.0	2.2	2.6	1.6	1.5	1.6	15.2	8.0	11.6	0.0	1.1	0.6
	H2	3.4	2.5	3.0	1.8	1.9	1.9	18.6	15.7	17.2	0.1	1.3	0.8
	H3	3.5	2.4	3.0	2.0	1.8	1.9	21.7	14.3	18.0	0.5	1.6	1.1
	H4	3.0	2.4	2.7	2.1	1.6	1.9	20.7	13.4	17.1	0.0	1.9	1.0
	H5	3.3	2.6	3.0	1.7	1.9	1.8	18.3	14.9	16.6	0.0	2.6	1.3
	H6	3.4	2.5	3.0	1.9	1.8	1.9	16.7	16.5	16.6	0.0	5.2	2.6
	H7	3.2	2.6	2.9	2.1	1.9	2.0	16.0	14.4	15.2	0.0	1.7	0.9
Mean		3.3	2.5	2.9	1.9	1.8	1.9	18.2	13.9	16.0	0.1	2.2	1.2
Kharif	H1	3.6	2.9	3.3	1.8	1.8	1.8	19.9	22.8	21.4	0.0	2.5	1.3
	H2	3.7	3.2	3.5	2.2	1.9	2.1	22.7	23.0	22.9	0.2	2.9	1.6
	H3	3.9	3.1	3.5	2.3	1.8	2.2	28.0	25.0	26.5	0.3	3.4	1.9
	H4	4.0	3.0	3.5	2.4	2.0	2.2	20.6	24.2	22.4	0.0	3.1	1.6
	H5	4.1	3.1	3.6	2.0	2.1	2.1	22.6	22.9	22.8	0.0	10.8	5.4
	H6	3.9	3.0	3.5	2.0	2.3	2.2	24.4	23.5	24.0	0.2	5.4	2.8
	H7	4.1	3.2	3.7	2.2	2.2	2.1	20.3	24.1	22.2	0.3	3.9	2.1
Mean		3.9	3.1	3.5	2.1	2.0	2.1	22.6	23.6	23.2	0.1	4.6	2.4
Overall mean		3.6	2.8	3.2	2.0	1.9	2.0	20.4	18.8	19.6	0.1	3.4	1.8

V = Vikas; L = Lohit; H1 = Control; H2 = GA_3 (50 ppm); H3 = GA_3 (100 ppm); H4 = IAA (50 ppm); H5 = IAA (100 ppm); H6 = GA_3 (50 ppm) + IAA (100 ppm); H7 = GA_3 (100 ppm) + IAA (50 ppm)

cross 'Vikas' × 'Lohit' in kharif after the application of 50 ppm IAA. Roupakiens (1986) also reported the slower growth of hybrid pods as compared to selfed pods in *Vicia*.

A good seed set was recorded in the case of selfing in both the spring and kharif seasons, with the highest seed set of 28 seeds/boll for 'Vikas' in the kharif season. Among the crosses maximum seed set (10.8 seeds/boll) was recorded for 'Lohit' × 'Vikas' in the kharif season (Table 4). In most of the hybrid bolls which grew normally till harvest, the seeds were very small and the embryos were aborted. The abortion of the embryo in interspecific crosses of *Gossypium* was reported by He and Liang (1989) and He et al. (1991).

Growth regulator application led to an increase in seeds/boll both in parents and crosses. The best growth regulator was found to be IAA at 100 ppm concentration followed by the combination GA_3 (50 ppm) + IAA (100 ppm) for hybrid seeds. An increase in the number of seeds after the application of growth regulators was reported by Bhale et al. (1987) and Altman (1988). Kodali and Khanna (1994) also reported the positive effect of gibberellic acid on seed set in wheat-barley crosses.

It is suggested that when making *G. hirsutum* × *G. arboreum* crosses, the growth regulator combination GA_3 (50 ppm) + IAA (100 ppm) or IAA 100 ppm alone should be sprayed during emasculation in the kharif season to obtain better boll and seed set.

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EFFECTS OF ELEVATED CO_2 CONCENTRATION ON THE DEVELOPMENT AND YIELD COMPONENTS OF CEREALS

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Fourteen cereal varieties (eight winter wheat varieties bred and grown in the major wheat growing zones of the world, together with one variety each of triticale, rye, barley, winter durum wheat, and winter and spring oats) were grown in two phytotron units at 375 and 750 $\mu mol \cdot mol^{-1}$ (ppm) CO_2 concentration to evaluate the effects of elevated CO_2 concentration on development and yield components, and to compare the responses of different cereal varieties. In the sixth week of the experiment the number of shoots, leaf area and canopy dry weight were measured and average increases of 75%, 75% and 50%, respectively, were observed for crops grown at 750 $\mu mol \cdot mol^{-1}$ CO_2 compared with those grown at normal CO_2 concentration. The extent of these differences varied, however, from one species to the other, ranging from 12–150%, 38–188% and 0.5–57%, respectively. The most sensitive was the winter wheat cultivar Libellula, while the triticale (cv. Presto) showed the smallest sensitivity to elevated CO_2 concentration during the first six weeks of the experiment. The increase in grain yield was connected very closely with an increase in ear number/plant and grain number/plant. With the exception of three varieties (two winter wheat varieties, cvs. Fredrick and Bezostaya 1, and the winter durum wheat, cv. Hordeiforme 1443) the grain yield was greater when plants were grown at 750 $\mu mol \cdot mol^{-1}$ CO_2 concentration. It was observed that the triticale, with the smallest responses to elevated CO_2 at the sixth week, showed the highest increase in grain yield (40%). The elevation of CO_2 concentration had no effect on the average thousand grain mass.

Key words: cereals, plant development, yield components, global climate changes, CO_2 concentration

Introduction

Over the past few decades the quantity of carbon dioxide emitted into the earth's atmosphere due to human activities has increased to such an extent that its concentration in the troposphere is now over 25% higher than it was prior to the industrial revolution (350–380 $\mu mol \cdot mol^{-1}$, compared with 280 $\mu mol \cdot mol^{-1}$). This represents a rise of some 0.45% a year, which can be measured on all parts of the globe (Keeling et al., 1976 a,b; Haszpra, 1995; Hofmann and Peterson, 1996).

Experiments have been carried out continuously since the seventies to analyse the effect of a rise in CO_2 concentration on wheat (Krenzer and Moss, 1975; Kendall et al., 1985; Mitchell et al., 1993; Nicolas et al., 1993; Wheeler et al., 1996). It was found that the photosynthesis of plants raised at higher CO_2 concentrations was more intense, while the evapotranspiration decreased compared to plants grown in a normal environment, leading to a greater biomass

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quantity and, in many cases, to higher yields. As the result of greater CO_2 concentration an increase of over 25% was demonstrated in the dry mass of the plants immediately prior to shooting (Neales and Nicholls, 1978), and the higher CO_2 concentration may also have a positive effect on tiller number, leaf number and leaf area (Gifford, 1977; Morison and Gifford, 1984; Batts et al., 1996). In other experiments it was observed that the increase in dry mass up to heading may be as great as 6–31% (Mitchell et al., 1995; Wheeler et al., 1996).

In the majority of cases increased CO_2 concentration had a positive effect on both the yield and the yield components. Many authors have recorded an increase in yield after shorter or longer treatments with CO_2 (Havelka et al., 1984; Mitchell et al., 1995; Wheeler et al., 1996), though a reduction in yield has also been noted (Tuba et al., 1994). This yield increase can be explained in various ways: Combe (1981) considered it to be caused by a greater number of spikelets per spike, Fischer and Aguilar (1976) put it down to a larger number of spikes per plant, thus giving a higher number of grains, while Krenzer and Moss (1975) and Sionit et al. (1981) attributed it to an increase in grain mass at higher CO_2 concentrations.

Investigations on the effect of high atmospheric CO_2 concentration on cereals have usually been limited to experiments on one or two winter wheat varieties commonly grown at the experimental site, while very little is known about the response to increased CO_2 concentration of other cereals, such as rye, barley, triticale and oats, which are also important crops. The aim of the present work was to examine under phytotronic conditions the development, biomass production and yield potential of winter wheat varieties and other cereals originating from production zones with various climates when grown at an atmospheric CO_2 concentration double that recorded at present.

Materials and methods

The examinations were made on winter wheat varieties originating from the eight major wheat-growing regions of the world (Fredrik – Canada, Karl – USA, Alba – Poland, Martonvásári 15 – Hungary, Libellula – Italy, Thesee – France, Bezostaya 1 – Russia, Gerek –Turkey) and on one variety each of durum wheat (Hordeiforme 1443), rye (Motto), barley (Kompolti korai), winter oats (Gerald), spring oats (Kwant) and triticale (Presto), all of which are cultivated in Hungary. Seed from the majority of the varieties was obtained from the variety collection of the institute's Wheat Breeding Department, while the remainder were provided by various breeding institutes (Gerald – Welsh Plant Breeding Station, Aberystwyth, UK; Kwant – Plant Breeding and Acclimatisation Institute, Radzikow, Poland).

The winter cereals were vernalised for seven weeks at 2°C and then grown in two Conviron PGV-36 plant growth chambers (Tischner et al., 1997), in one of which the CO_2 concentration was normal (375 $\mu mol \cdot mol^{-1}$), while in the other this value was doubled (750 $\mu mol \cdot mol^{-1}$). The climatic conditions in both chambers were similar to the average spring and summer weather conditions in Hungary (Table 1).

Table 1
Growing conditions during the experiment

Weeks	T_{max} (°C)	T_{min} (°C)	Daylength (h)	PPFD* (μmol/m^{2}s)
1–2	14	10	13.5	280
3	15	11	14.0	280
4–5	16	12	15.0	280
6–7	17	13	15.5	280
8–13	18	14	16.0	340
14	19	15	16.0	440
15	20	16	16.0	440
16	21	17	15.5	440
17	22	18	15.5	440
18	23	19	15.5	440
19	24	20	15.5	440

*Photosynthetic Photon Flux Density

The plants were grown in chernozem soil with forest residues and a good nutrient supply, with 4 plants in each of four 18 cm x 18 cm × 18 cm plastic pots per variety and treatment. The plants were watered daily, supplemented twice a week alternately with Wuxal (concentration in water: 0.05–0.1%; contents: N – 8, P_2O_2 – 8, K_2O – 6 m/m%; Fe – 150, Mn – 130, Mo – 10, B – 100, Zn – 50, Cu – 70 mg/kg) and Volldünger (concentration in water: 0.3%; contents: N – 14, P_2O_2 – 7, K_2O – 21, MgO – 1, Zn –1, Fe –1, Mn –1, Cu –1, B – 1 m/m%) solution. Both at shooting and immediately prior to heading, they were watered on two occasions with a nutrient solution prepared from complex fertiliser (N – 16, P_2O_5 – 16, K_2O – 16 m/m%). In this way a satisfactory nutrient supply was ensured for the plants throughout the experiment.

The developmental state of the plants was noted once a week, involving measurements of plant height and number of tillers; the dates of shooting and heading were also recorded. In the 6th week of the experiment the plant height, number of tillers, leaf area (leaf area meter type AAM-7, Hayashi Denkoh Co. Ltd., Tokyo) and above- and belowground dry mass (after drying for 24 hours at 105°C) were recorded for 4 plants of each variety in each treatment, i.e. one plant from each pot. The plants were harvested in the 20th week of the experiment, when all the plants were physiologically mature, and measurements were made on the number of spikes, number of grains, grain mass and stem mass of the plants.

The effect of increased CO_2 concentration was analysed using two-way analysis of variance, while the correlation between the yield components was examined by means of regression analysis.

Results and discussion

Early developmental phase

In the early developmental phase all the cereals examined responded positively to additional CO_2. This is indicated by the substantial increase in number of tillers, leaf area and dry mass observed by the sixth week (Table 2). The number of tillers doubled in most of the varieties; the leaf area increased by an average of 50%, and the aboveground dry mass by 75% compared with the control plants. This increase exceeds that in previous data published for the early

Table 2
Shoot number, canopy dry weight and leaf area of six-week-old cereal plants grown at 375 or 750 $\mu mol \cdot mol^{-1}$ CO_2 concentration

Cereal varieties	No. of shoots/plant at		Canopy dry weight (g/plant) at		Leaf area (cm^2/plant) at	
	375	750	375	750	375	750
	$\mu mol \cdot mol^{-1}$ CO_2 concentration		$\mu mol \cdot mol^{-1}$ CO_2 concentration		$\mu mol \cdot mol^{-1}$ CO_2 concentration	
Winter wheat varieties						
Fredrick	3.00	6.00***	0.39	0.72*	76.98	121.06*
Karl	2.67	7.00***	0.24	0.48	50.17	87.01
Alba	2.67	6.67***	0.30	0.55	60.75	100.61*
Mv 15	2.67	6.00***	0.26	0.47	48.72	94.66*
Libellula	3.00	6.67***	0.45	0.81*	82.15	144.22**
Thesee	3.00	5.33**	0.37	0.65*	83.55	143.41**
Bezostaya 1	3.00	6.00***	0.42	0.62	92.71	121.74
Gerek	3.67	5.00	0.36	0.51	82.42	110.68
Triticale						
Presto	5.33	6.00	0.63	0.87	131.70	138.20
Rye						
Motto	6.33	8.33*	0.64	0.88	154.25	181.40
Winter barley						
Kompolti korai	3.00	5.00*	0.34	0.92***	94.45	153.89**
Winter oats						
Gerald	2.67	4.33	0.22	0.47	61.84	98.88*
Winter durum wheat						
Hordeiforme 1443	3.33	6.00**	0.36	0.72**	71.52	113.48*
Spring oats						
Kwant	2.00	3.33	0.32	0.71**	65.84	108.94*
Mean	3.31	5.83*	0.38	0.67*	82.65	122.73*
$LSD_{5\%}$	1.7		0.26		37	
$LSD_{5\%}$ (treatment mean)	0.45		0.07		9.88	

*, **,*** Significant at the 0.05, 0.01 and 0.001 probability levels, respectively

stage of development (Neales and Nicholls, 1978; Batts et al., 1996). The increases were statistically non-significant in two of the lines examined (the winter wheat variety Gerek and the triticale variety Presto). Changes were observed in the ratio of the aboveground to belowground dry matter as the result of the treatment in spring oats (Kwant) and triticale (Presto). This ratio generally increased from 5.3 to 8.9 and from 4.8 to 6.7. For the other varieties the ratio of aboveground to belowground parts was the same in both chambers, ranging from 2.9 to 6.3 depending on the variety. The initial substantial increase in biomass was later reflected in the differences in plant height recorded as the result of increased CO_2 concentration for all the varieties by the 6th week of treatment, which was as great as 15 cm by the 10^{th} week (Fig. 1).

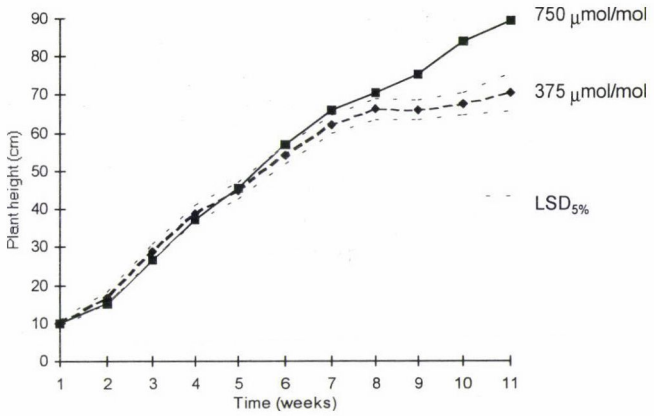

Fig. 1. Height of cereal plants averaged over 14 varieties at normal and elevated atmospheric CO_2 concentration. $LSD_{5\%}$ was calculated for each week separately

Duration of development

No difference was observed for the shooting date, but the beginning of heading was influenced by the treatment in the case of six varieties (Table 3). Plants grown in both the control chamber and at increased CO_2 concentration began to shoot after 47±5 days, averaged over the varieties. The only variety to exhibit a significant difference was the winter wheat Bezostaya 1, which began to shoot 3 days earlier at increased CO_2 concentration than in the control treatment. Six varieties (the winter wheat cvs. Bezostaya 1 and Martonvásári 15, the rye variety Motto, the winter oat variety Gerald, the winter barley variety Kompolti korai and the spring oat variety Kwant) headed 3–8 days earlier at increased CO_2 concentration than under normal conditions. The other six winter wheats and the winter durum wheat headed at the same time in both chambers, after 70±5 days, averaged over the varieties. In the literature no changes attributable to the effect of increased CO_2 concentration are noted in the dates when various developmental stages are reached (Batts et al., 1996; Wheeler et al., 1996). In the present studies this was true for the majority of plants examined.

Yield parameters

When averaged over the 14 varieties examined, plants raised at a CO_2 concentration of 750 µmol· mol^{-1} produced grain yields significantly higher at the 5% level of significance, but for the individual varieties significant yield increases compared to the control were only found for the winter wheat Alba, the triticale Presto and the winter barley Kompolti korai, while the increase in yield in the other varieties was statistically non-significant (Table 4).

Table 3
Duration of growth from the beginning of the experiment to tillering and heading in cereal plants grown at 375 or 750 $\mu mol \cdot mol^{-1}$ CO_2 concentration

Cereal varieties	Days to shooting at		Days to heading at	
	375	750	375	750
	$\mu mol \cdot mol^{-1}$ CO_2 concentration		$\mu mol \cdot mol^{-1}$ CO_2 concentration	
Winter wheat varieties				
Fredrick	50	48	71	71
Karl	44	43	60	60
Alba	55	56	81	81
Mv 15	46	45	73	69**
Libellula	41	40	58	58
Thesee	49	47	68	67
Bezostaya 1	46	43*	68	65**
Gerek	45	46	67	66
Triticale				
Presto	49	50	69	69
Rye				
Motto	#	#	86	80***
Winter barley				
Kompolti korai	45	45	67	64**
Winter oats				
Gerald	52	51	83	75***
Winter durum wheat				
Hordeiforme 1443	42	43	68	67
Spring oats				
Kwant	42	42	69	65***
Mean	48	47	71	68*
$LSD_{5\%}$	2.5		2.2	
$LSD_{5\%}$ (treatment mean)	1.2		0.6	

*, **, *** Significant at the 0.05, 0.01 and 0.001 probability levels, respectively
No data available

Increases in number of grains were statistically significant for seven of the fourteen varieties and the correlation coefficient between grain yield and number of grains was 0.89 (P = 5%). The thousand grain mass increased significantly in only three winter wheat varieties (Alba, Martonvásári 15 and Thesee). The number of spikes increased significantly at increased CO_2 concentration when averaged over the varieties. These results are consistent with other published data for various varieties.

Correlation between vegetative biomass and yield parameters

Immediately prior to shooting an average biomass increase of 75% could be recorded at 750 $\mu mol \cdot mol^{-1}$ CO_2 concentration compared to the control. The

Table 4
Yield components of cereal plants grown at 375 or 750 $\mu mol \cdot mol^{-1}$ CO_2 concentration

Cereal varieties	Grain yield (g/plant) at		No. of grains/plant at		Thousand grain mass (g) at		No. of ears/plant at	
	375	750	375	750	375	750	375	750
	$\mu mol \cdot mol^{-1}$ CO_2 concentration		$\mu mol \cdot mol^{-1}$ CO_2 concentration		$\mu mol \cdot mol^{-1}$ CO_2 concentration		$\mu mol \cdot mol^{-1}$ CO_2 concentration	
Winter wheat varieties								
Fredrick	3.28	2.76	88.31	83.19	37.31	33.51	2.19	2.69
Karl	2.30	2.52	74.81	76.00	31.11	33.46	3.00	3.75*
Alba	3.36	4.82**	112.63	132.56*	30.13	37.41*	2.06	3.25**
Mv 15	3.08	3.93	92.25	96.67	33.50	40.42*	1.81	2.47
Libellula	3.55	4.02	87.88	95.13	40.23	42.08	2.88	3.44
Thesee	3.19	3.71	86.87	83.06	36.73	44.66*	2.07	2.31
Bezostaya 1	2.95	2.66	64.44	55.31	46.22	48.09	2.38	3.44**
Gerek	3.25	4.25	78.44	111.00**	41.77	38.06	2.44	3.20*
Triticale								
Presto	3.50	4.99**	84.13	127.13***	43.90	41.14	1.94	3.50***
Rye								
Motto	0.81	1.54	25.80	50.10*	33.46	30.75	2.10	2.80
Winter barley								
Kompolti korai	4.31	6.56**	92.75	144.80***	46.06	45.38	2.75	3.87**
Winter oats								
Gerald	4.37	5.29	137.27	175.00***	31.68	29.98	2.20	2.81
Winter durum wheat								
Hordeiforme 1443	2.09	2.02	58.88	49.31	35.45	40.73	1.69	2.00
Spring oats								
Kwant	4.12	4.73	128.75	168.94***	32.01	28.15	2.44	2.31
Mean	3.15	3.84*	86.66	103.44*	37.11	38.13	2.28	2.99*
$LSD_{5\%}$	1.11		20.09		6.52		0.72	
$LSD_{5\%}$ (treatment mean)	0.29		8.28		1.74		0.19	

*, **,*** Significant at the 0.05, 0.01 and 0.001 probability levels, respectively

yield, however, increased by only 20% over the average of the varieties and correlation studies reveal that the biomass increase observed by the sixth week of treatment had little or no influence on the yield. A good example of this is the winter wheat variety Fredrick, where the biomass was almost doubled at 6 weeks, yet the yield did not increase at increased CO_2 concentration. In contrast, the triticale variety Presto and the winter wheat variety Gerek exhibited no significant difference in development by the sixth week of the treatment compared with the control, but produced significantly higher yields at increased CO_2 concentration.

The number of tillers, which doubled during the initial stages of development compared with that of the control plants, did not change at the same rate up to heading, so the number of spikes was only 30% greater on average for plants grown at increased CO_2 concentration than for those grown at normal CO_2 concentration. These results are in agreement with those reported by Batts et al. (1996).

The ratio of yield to stem mass changed significantly as the result of increased CO_2 concentration, dropping on average by 25%, from 2.05 to 1.52, reflecting a smaller increase in yield than in stems. As a consequence the harvest index was reduced at increased CO_2 concentration. This change is roughly equivalent to that reported by Tuba et al. (1994), but contradicts the results published by Wheeler et al. (1996), who found that this ratio did not change as the result of the treatment.

Evaluation of individual varieties

All the 14 varieties examined showed a significant change in some respect as the result of the treatment. In the initial stage of development the varieties exhibited very similar responses to increased CO_2 concentration. A substantial increase in biomass production was observed for all except the triticale (Presto), where the positive response appeared somewhat later. This similarity in the response of the species and varieties was lost by the beginning of heading, as indicated by the reduction in the days to heading for two winter wheat varieties (Mv 15 and Bezostaya 1), and for the rye, winter barley, and winter and spring oat varieties.

Differences in the yield parameters were observed between the varieties, which can, therefore, be divided into three major groups. The largest group is formed by the six varieties (Libellula, Gerek, Presto, Motto, Kompolti korai, Gerald) which produced more spikes and thus had a higher number of grains and yield, while their thousand grain mass decreased or showed no change. The second group consists of two winter wheat varieties (Alba, Mv 15), where the increase in yield was due to a joint increase in thousand grain mass, spike number and grain number. In the third group (Fredrick, Karl, Thesee, Bezostaya 1, Kwant, Hordeiforme 1443) there was either no change or a slight reduction in yield. Although most of the varieties in this group exhibited a rise in one of the yield parameters in response to increased CO_2 concentration, this was compensated by a drop in the other two parameters, so there was no increase in yield.

The response to the CO_2 treatment differed for each species and variety. Varieties were found (e.g. Alba) with a uniformly positive response. There were also varieties (e.g. Fredrick) with an initial substantial increase followed by a reduction in yield, and the opposite (Presto). The responses of the eight winter

wheat varieties to higher CO_2 concentration were not more closely correlated to each other than to those of the other cereals.

It can be concluded from the results that the ability of cereal species to use higher CO_2 concentrations depends on the genetic background rather than on the site of origin. The differences in the extent of the generally positive plant responses to increased CO_2 concentration in various development phases varied not only between the cereal species, but also between the varieties within the winter wheat species.

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INTERRELATION BETWEEN NITROGEN STATUS OF THE PLANTS, LEAF CHLOROPHYLL AND GRAIN YIELD IN VARIOUS WINTER WHEAT CULTIVARS

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The relations between chlorophyll and nitrogen in the leaves and grain yield of various winter wheat cultivars were investigated. The leaf chlorophyll content in plants at the same growth stage was in close correlation with leaf nitrogen content and grain yield. During the second half of vegetation a positive correlation was observed between yield and total chlorophyll content in the plants among cultivars with different maturing rates. The usefulness of different chlorophyll parameters for the remote sensing of plant status and yield prognosis in the crops of various winter wheat cultivars is estimated.

Key words: winter wheat, cultivars, nitrogen, chlorophyll, grain yield

Introduction

The problems of the rapid diagnosis of nitrogen status and yield prediction in grain crops necessitate a search for parameters indicative of plant status and the elaboration of precise, convenient methods for measuring these.

Much attention is paid to crop estimation, based on the dependence between soil and leaf nitrogen on the one hand, and between leaf chlorophyll content and grain yield on the other (Andreyeva and Voevudskaya, 1992; Blackmer et al., 1994; Costa, 1991; Follett et al., 1992; Grover et al., 1978; Kochubey et al., 1988a; Overman et al., 1994; Piekielek et al., 1995; Schepers et al., 1992).

Chlorophyll indexes, which characterize the potential power of the photosynthetic apparatus, can be estimated with the help of spectral methods (Hoffman et al., 1986; Kochubey et al., 1988b; Takebe et al., 1990; Thomas and Oerther, 1972). Therefore, there is a possibility for the objective remote sensing of the status of farm crops on large areas within a real time scale (Shadchina and Kochubey, 1992; Wood et al., 1992; Shibayama and Akiyama, 1986).

The practical implementation of this idea requires the solution of a number of scientific and methodological problems which will determine the quantity and quality of the information obtained. One such problem is the effect of winter wheat cultivar peculiarities on both the estimation of plant nitrogen nutrition and the yield prognosis based on leaf diagnostic data.

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As far as the nitrogen content determination in winter wheat leaves by their spectral characteristics in the visual region is conducted via chlorophyll, the true nitrogen estimation will depend on the stability of the chlorophyll-nitrogen relation. At present, there is not sufficient information concerning cultivar–dependent peculiarities in the chlorophyll–nitrogen relation in winter wheat leaves. The question of the interrelationship between chlorophyll parameters and grain yield has also to be solved.

The objective was to study the interrelationship between leaf nitrogen and chlorophyll contents and between different chlorophyll parameters and grain yield for a number of winter wheat cultivars differing in phytometric characteristics, chlorophyll levels and vegetation duration.

Materials and methods

Twenty-four winter wheat (*Triticum aestivum*) cultivars belonging to three groups (early-ripening, medium-ripening and late-ripening) were investigated. The early-ripening group comprised: Mironovskaya bearded, Donskoy semidwarf, Kievskaya bearded, Yubileinaya-75 and Mironovskaya-29. The medium-ripening group comprised: Zbruch, Spartanka, Albatros Odesskiy, Mironovskaya-27, Mironovskaya-61, Mironovskaya-808, UK-17, UK-115, Ivanovskaya-60, Lutescence-7, Aisberg, Briz, Fedorovka, Mirleben and MV-17. The late-ripening group comprised: UK-10, UK-21, UK-31 and UK-205. The plants were grown on test plots on the "Glevakha" experimental farm of the Institute of Plant Physiology and Genetics. Nitrogen fertilizer (60 kg of active substance per ha) was applied in the booting stage. Total nitrogen content was determined by the Kjeldahl method modified after Pochinok (1976). Leaf chlorophyll content was estimated according to Arnon (1949). The experimental results were evaluated by statistical methods according to Lakin (1980).

Results and discussion

The results of chlorophyll (Chl) and nitrogen (N) determination in the leaves of different winter wheat cultivars at the same dates during vegetation (1993 and 1994) are presented in Tables 1 and 2. The plants are grouped by the stage of vegetation for each date. The data of the experiment caried out in 1993 are listed for early- and medium-ripening cultivars from the milky and milky-waxy maturity (24.06.93) to milky-waxy and waxy maturity (5.07.93) stages of the grain. This period covers the second half of the vegetation period. As the leaves began to senesce, the chlorophyll content decreased simultaneously with the nitrogen content. The dependence between these parameters was described by a linear function with a correlation coefficient of $r = 0.83 \pm 0.11$ (Fig. 1).

In 1994 the observation period for the Chl and N contents in the leaves of medium- and late-ripening cultivars was from the booting stage to the milky-waxy stage of grain maturity (Table 2). In the first half of the vegetation period, when chlorophyll is accumulated in the leaves, the dynamics of nitrogen content

Table 1
Leaf chlorophyll and nitrogen contents, and grain yield per stem in various winter wheat cultivars (1993)

Date	Cultivar	Phase	Chlorophyll in flag leaves (mg dm^{-2})	N in flag leaves (% of abs. dry matter)	Grain yield (g)
24.06.93.	*Mid-ripening*	Milk maturity			
	Spartanka		7.65±0.11	4.26±0.04	–*
	Albatros Odesskyi		6.90±0.52	4.42±0.01	0.81
	Mironovskaya-61		7.74±0.03	4.42±0.01	0.84
	Zbruch		9.22±1.05	4.26±0.01	1.03
	Mironovskaya-27		7.56±0.16	4.04±0.62	0.85
	MV-17		6.60±0.03	4.04±0.02	0.93
	Lutescence-7		7.06±0.22	4.20±0.03	
	Lutescence-7		6.69±0.52	4.08±0.02	–
	Lutescence-7		6.32±0.43	4.01±0.02	–
	Mironovskaya-808		5.88+0.21	3.84+0.01	–
	Polesskaya -70		6.16+0.18	3.86+0.02	–
	Early ripening				
	Kievskaya bearded		7.02±0,40	4.10±0,01	0.81
	Mironovskaya-29		7.59±0.35	3.90±0.03	0.83
	Yubileinaya-75		7.42±0,91	3.97±0.03	0.81
	Donskoi semidwarf		6.13±0.44	3.58±0.01	0.77
06.07.93	*Mid-ripening*	Milky waxy			
	Spartanka		5.68±0.01	3.22±0.03	–
	Albatros odesskyi		5.35±0.37	3.10±0.02	0.81
	Mironovskaya-61		7.89±0.22	3.45±0.01	0.84
	Zbruch		7.20±0.12	3.77±0.01	1.03
	Mironovskaya-27		6.63±0.23	3.31±0.01	0.85
	MV-17		6.00±0.17	3.39±0.03	0.93
	Lutescence-7		4.18+0.15	2.79+0.02	–
	Mironovskaya-808		4.74+0.31	3.02+0.01	–
	Polesskaya-70		4.35+0.27	2.91+0.02	–
	Early ripening	Beginning			
	Kievskaya bearded	of waxy	4.91±0.07	3.27±0.02	0.81
	Mironovskaya-29	maturity	4.09±0.09	2.88±0.04	0.83
	Yubileinaya-75	of the grain	6.17±0.12	3.17±0.02	0.81
	Donskoi semidwarf		3.26±0.06	2.64±0.02	0.77

* not estimated

depends on the soil nitrogen. In the absence of nitrogen fertilization, the leaf nitrogen content decreases as a rule. In the present case, the spring application of fertilizer as side-dressing significantly slowed down the reduction in nitrogen content.

Table 2
Leaf chlorophyll and nitrogen contents, and grain yield per stem in various winter wheat cultivars (1994)

Date	Cultivar	Phase	Chlorophyll in flag leaves (mg dm^{-2})	N in flag leaves (% of abs. dry matter)	Grain yield (g)
01.06.94.	*Mid-ripening*	heading			
	Mironovskaya bearded		7.31±0.30	4.53±0.01	0.92
	Mironovskaya-61		7.79±0.17	4.75±0.04	1.20
	Zbruch		8.06±0.17	4.77±0.03	0.94
	Albatros Odesskyi		8.43±0.57	4.88±0.02	0.88
	UK-115		8.24±0.02	4.93±0.03	1.25
	Mirleben		6.69±0.08	4.69±0.02	1.50
	Late ripening	booting			
	UK-205		7.31±0.30	4.75±0.01	1.26
	UK-10		7.20±0.12	4.53±0.01	1.43
	UK-21		7.62±0.31	4.86±0.01	1.32
	UK-31		6.70±0.03	4.69±0.03	0.95
14.06.94	Mironovskaya bearded	Completion of	8.06±0.34	4.42±0.01	0.92
	Mironovskaya-61	flowering	9.22±0.37	4.67±0.01	1.20
	Zbruch		8.77±0.37	4.77±0.01	0.94
	Albatros Odesskyi		9.14±0.01	4.83±0.02	0.88
	UK-115		8.40±0.10	4.86±0.02	1.25
	Mirleben		8.42±0.14	4.46±0.02	1.50
	UK-205	flowering	8.57±0.68	4.59±0.01	1.26
	UK-10		8.85±0.74	4.51±0.01	1.43
	UK-21		8.28±0.29	4.70±0.03	1.32
	UK-31		8.83±0.43	4.68±0.01	0.95
05.07.94.	Mironovskaya bearded	milky	7.41±0.10	3.90±0.02	0.92
	Mironovskaya-61	waxy	8.88±0.73	3.80±0.02	1.20
	Zbruch		9.94±0.42	4.28±0.01	0.94
	Albatros Odesskii		8.05±0.01	3.96±0.01	0.88
	UK-115		9.45±0.30	4.18±0.01	1.25
	Mirleben		8.54±0.30	3.68±0.01	1.50
	UK-205	milky	8.69±0.15	4.00±0.03	1.26
	UK-10		9.61±0.15	4.07±0.01	1.43
	UK-21		7.84±0.21	3.93±0.02	1.32
	UK-31		7.99±0.01	3.83±0.02	0.95

* not estimated

Within each season, the plants of various cultivars grown under identical weather and nutrition conditions could be distinguished by their leaf chlorophyll levels. At the same dates, these distinctions were observed not only for cultivars with different vegetation durations, but also for plants with the same ripening rates. The increased nitrogen level for some cultivars may be explained by their higher activity in taking up nitrogen from the soil.

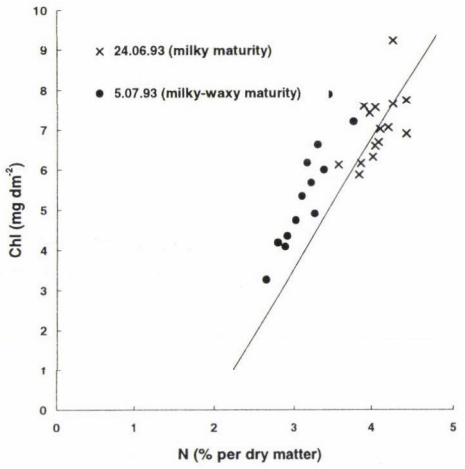

Fig. 1. Dependence between chlorophyll and nitrogen content in flag leaves of various winter wheat cultivars in the phases of milky and milky-waxy maturity (1993 data, see Table 1)

There is a positive correlation between chlorophyll and leaf nitrogen content for plants of different cultivars in the same vegetation stage. The dependences between leaf chlorophyll and nitrogen content for various cultivars in the booting (curve 1), heading (curve 2) and flowering stage (curve 3) and from milky to milky-waxy maturity (curve 4) are presented in Figure 2.

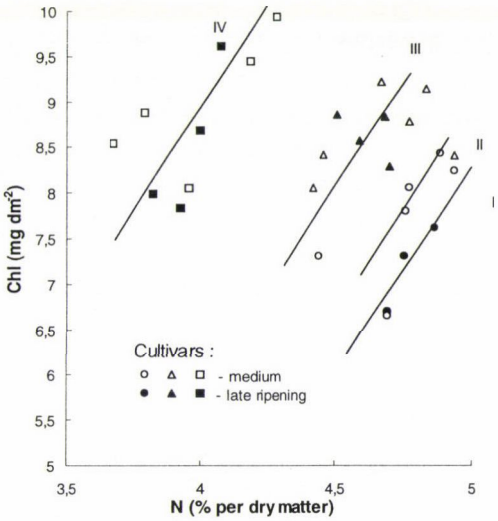

Fig. 2. Dependence between chlorophyll and nitrogen content in flag leaves of various winter wheat cultivars in different phases of vegetation (1994 data, see Table 2). I – booting, II – flowering, III – completion of flowering, IV – milky to milky–waxy grain maturity

The points of curve 1 correspond to the late-ripening cultivars which were in the booting stage (1.06.94), while the plants of medium-ripening cultivars were at the heading stage. The data on chlorophyll and nitrogen content in the leaves of medium-ripening cultivrs show the dependence described by curve 2. On 14.06.94 the plants were at the flowering stage (late-ripening cultivars) or at the completion of flowering (medium-ripening cultivars). In this period, as a rule, the chlorophyll accumulation in the flag leaves reaches its maximum level. The flag leaves in late-ripening cultivars were characterized by lower chlorophyll contents due to their younger age. Altogether the data for the cultivars of both groups are a set of correlation field points which may be described with a certain accuracy by a common linear function (curve 3).

On 5.07.94 the plants of winter wheat were at the stage of milky and milky-waxy maturity. In this case the interrelationship between chlorophyll and nitrogen content was retained, but with a lower leaf nitrogen level in comparison with the preceding stages.

The results presented provide evidence that at each stage a linear dependence between the nitrogen and chlorophyll contents in the leaves is preserved. This is also confirmed by the results of the three-year research (1991, 1993, 1994) on various winter wheat cultivars at the stage of milky-waxy maturity (Fig. 3). All these data are described by a linear regression function with a correlation coefficient of $r = 0.95 \pm 0.05$. Such a close correlation reveals the identical nature of the chlorophyll-nitrogen interrelationship for various cultivars. The level of leaf chlorophyll for plants within a certain cultivar is known to be closely correlated with the leaf surface area and the grain yield. In the present research with different cultivars such a correlation was not revealed at the observation periods chosen, which could be explained by the different ripening stages of the investigated cultivars. However, even for plants in the same vegetation stages a significant positive correlation between the two parameters was not always observed. The correlation coefficient between yield and chlorophyll content for early- and medium-ripening cultivars was $r = 0.51$, while for the medium- and late-ripening cultivars (1994) no positive correlation was observed. If differences in the leaf surface areas of different cultivars were taken into account when calculating plant total chlorophyll, a positive correlation was revealed between grain yield and this integral parameter for cultivars in the same stage of vegetation (Fig. 4).

A positive correlation between grain yield and total chlorophyll content during the second half of vegetation was observed for all the cultivars studied (Fig. 5).

The correlation observed between the chlorophyll parameters and the leaf nitrogen and grain yield for various winter wheat cultivars supports the use of the chlorophyll parameters as an informative base in the remote sensing of the crops for the estimation of plant nitrogen status and yield prediction.

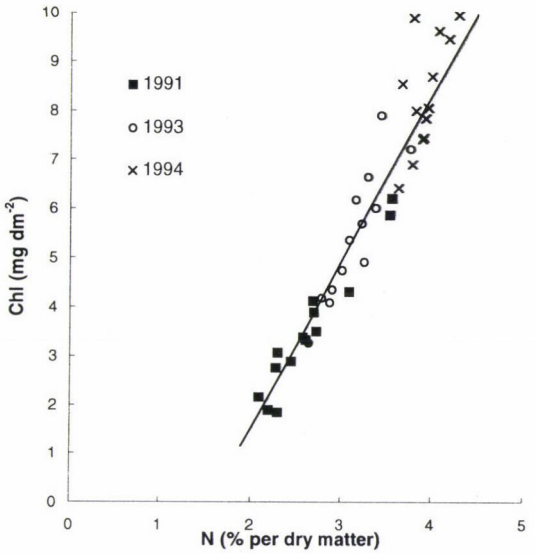

Fig. 3. Dependence between chlorophyll and nitrogen content in flag leaves of various winter wheat cultivars in phase milk-waxy grain maturity (Data of three years – 1991, 1993 and 1994)

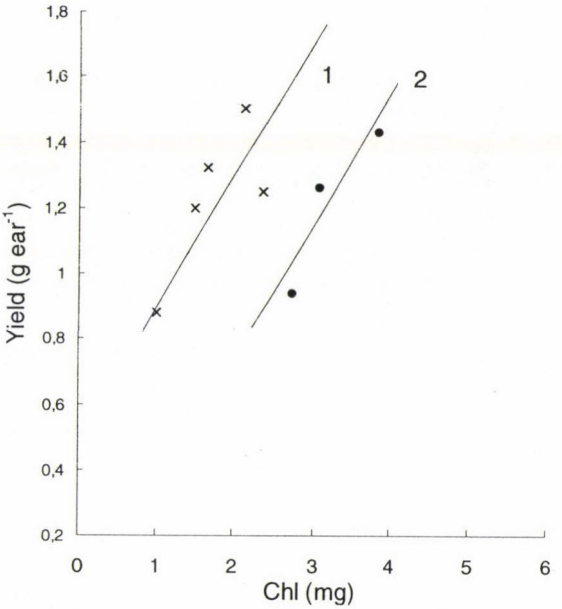

Fig. 4. Dependence between the total chlorophyll in all leaves of plants of late-ripening and medium-ripening winter wheat cultivars in phases of milky (2) and milky-waxy (1) maturity and the grain yield. (1994 data)

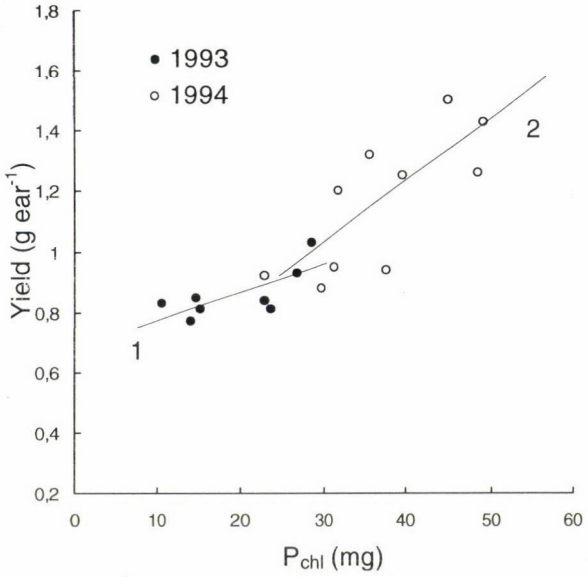

Fig. 5. Dependence between the chlorophyll potential calculated per stem in the plants of various winter wheat cultivars during the period from milky to milky-waxy maturity and the grain yield. 1. 1993 data; 2. 1994 data

The general character of the chlorophyll–nitrogen interrelationship for various cultivars allows leaf nitrogen to be estimated from the results of chlorophyll concentration independent of the cultivar.

When estimating the soil nitrogen status using leaf diagnostics it is necessary to take into consideration the peculiarities of the cultivars, which accumulate different levels of chlorophyll under identical agro-climatic conditions. This conclusion is in accordance with that of Schepers (1992) with respect to maize hybrids. According to his results, the normalization procedures should be used so as to standardize the tissue testing approach relative to hybrid type and location as well as to growth stages.

Since the relationship between chlorophyll and nitrogen content in plant leaves and soil depends on the climatic conditions, the nitrogen form and many other reasons (Shadchina and Dmitrieva, 1995; Patel and Khatri, 1983; Silberbush and Lips, 1991), the determination of soil nitrogen using leaf diagnostics seems to be very complicated.

The chlorophyll-nitrogen relationship in winter wheat leaves makes it possible to range yields within a cultivar by means of remote sensing. To predict the yield for different cultivars, it is necessary to measure the chlorophyll photosynthetic potential, i.e. the chlorophyll content in the crop during the vegetation period. The longer the observation period, the more accurate the

prediction. In addition, as the realization of the photosynthetic potential depends on the sensitivity of the cultivars to various stresses, the definition of photosynthetic efficiency may be useful for more precise yield prediction.

Thus, chlorophyll parameters can provide valuable information concerning plant nitrogen status and yield prognosis for various winter wheat cultivars. Leaf nitrogen content may be estimated by chlorophyll content using a regression equation suitable for all the cultivars investigated. Cultivar peculiarities should be considered when measuring the soil nitrogen status. It is also necessary to check the plant growth phase in this case. The chlorophyll photosynthetic potential during plant vegetation is more informative for estimating the yield of different winter wheat cultivars.

Conclusions

1. Leaf chlorophyll content is an informative parameter in the estimation of the nitrogen content in the leaves.
2. The interrelationship between leaf chlorophyll and nitrogen content is independent of the cultivar in winter wheat.
3. Yield prognosis can be performed on the basis of total chlorophyll in the crop during vegetation.

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ROOTS, PHOSPHORUS UPTAKE AND WATER USE EFFICIENCY OF MAIZE GENOTYPES

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The growth, phosphorus uptake and water use of six maize genotypes were studied in pot experiments in a controlled environment. Morphological and ecophysiological parameters of the genotypes, such as RWR (root weight ratio), SRL (specific root length), SLA (specific leaf area), P/RA (shoot P/root surface area), Lv (root length density), WUE (water use efficiency) and S (a stress factor on the relative growth rate basis) were studied. Genotypes with a more developed root system gave a better performance in water- and P-deficient conditions. Water use efficiency increased with increasing P supply. The hybrids Norma and Mv 444 and the inbred line Norma A were more efficient in water use and P acquisition than the lines Norma B, 444 A and 444 B. In respect of stress tolerance Norma was superior in the Norma-group as reflected in stress factors estimated on the relative growth rate (RGR) basis.

Key words: maize genotypes, roots, P acquisition, water use efficiency (WUE), morphological and ecophysiological parameters, controlled environment

Introduction

Water, N and P deficiencies are the most serious constraints on maize growth on high-yielding arable lands in Hungary. In the Danubian Basin, evapotranspiration is usually higher than precipitation. During the last two decades precipitation has decreased, while there was a trend towards increased evapotranspiration in the same period (Simonffy and Márton, 1995). The frequency of extremely dry periods both in summer and winter has increased. In consequence, arable soils may be depleted of water after harvest without replenishment by winter precipitation. This increases the risk of drought stress for the subsequent crop (Végh and Füleky, 1995). Unfortunately, irrigation water that can be used for crop production is only available in a few regions. At the same time surface and subsurface water resources are decreasing (Várallyay, 1993).

As a result of privatisation and the creation of small private farms, the consumption of fertiliser has dropped to one-third of that in the eighties (Láng, 1993).

Water and nutrient deficiencies are highly interactive, since a better water supply increases the total P uptake of maize (Szlovák, 1995), whereas the

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increased efficiency of the soil nutrients contributes to the most efficient use of available water. The morphology, density distribution and activity of the root system are considered to be important factors that enhance the availability of N and P. Furthermore, the distribution of nutrients in plants and their utilisation in metabolism and growth are considered to be the basis of genotypic differences in plant adaptation to nutrient and water deficiency stress.

The use of drought-tolerant cultivars, in conjunction with adequate fertilisation, is the only way to produce high maize yields without adverse effects on the environment. For efficient breeding programmes and also for proper water and nutrient management practices it is essential to understand the mechanisms which determine the ability of plants to perform satisfactorily in drought-prone areas. There is little published research on water × phosphorus × cultivar effects on the growth and nutrient uptake of maize, especially on Hungarian genotypes.

This study was conducted to determine the genotype-dependent response to soil water and P conditions in maize root growth and morphology, and to characterise the genotypic differences in morphological and ecophysiological traits during vegetative growth under water and phosphorus stress and non-stress conditions.

Materials and methods

Six genotypes were selected for this study: two hybrids, 'Norma' and 'Mv 444', and their four parental inbred lines: Norma A and Norma B, 444 A and 444 B. Norma is an early-maturing, productive hybrid with high drought tolerance and nutrient use efficiency. The hybrid Mv 444 makes efficient use of nutrients and has high yield potential. The plants were raised in pots containing 8 kg phosphorus-deficient sandy soil in a PGB-96 Conviron plant growth unit on a climatic programme elaborated for the growth of a generation (Tischner et al., 1997, Szk climatic programme). The light intensity (photosynthetic photon flux density) was 250–300 μmol m^{-2} s^{-1}, with a 16-hour light cycle; the daily minimum air temperature was set to 15°C and 16°C at the beginning and end of the experiment, respectively, and the maximum to 19°C and 24°C.

Three soil phosphorus levels (P1 = 0 mg, P2 = 25 mg, P3 = 250 mg P kg^{-1} soil) were adjusted by mixing P fertiliser into a sandy soil (pH 7.1, organic matter content 1.10%). The phosphorus fertiliser was thoroughly mixed into the soil. N, K and other nutrients were applied to the pots weekly in a phosphate-free macro + micronutrient solution. The soil water holding capacity was determined as a function of the suction of the soil, and soil moisture contents were adjusted to pF 2.7 (= D) and pF 2.0 (= M) pressure head, and maintained during the experiment. Evapotranspiration was monitored by using TDR sensors buried in the pots.

Six- and 8-week-old plants (6–8-leaf and 8–10-leaf stage, respectively) were harvested. The leaves and stalks were separated, and the biomass and leaf area (LA cm^2) were measured. The aboveground parts were then dried at 80°C, and the N and P contents of the leaves and stalk were determined after wet digestion.

Stress factors (S) were calculated on the relative growth rate (RGR) basis as:

$$S = 100(RGR_{max} - RGR)/RGR_{max}$$

A wet separation method was used to obtain the whole root system from the pots. The living and dead roots originating from the experimental plant were separated from the other crop residues contained in the soil by flotation and hand picking. Excess water was eliminated using a blotter and the root samples were weighed. After a two-day drying period the air-dry root samples were weighed again. Fresh weight (V) was used for estimating mean root radius. Root length (L) was determined by a line intersect counting method (Tennant, 1975). For P/RA calculations, the root surface area (RA) was estimated from the relation $RA = 2r_0\pi L$, where $r_0 = (V_r/L\pi)^{0.5}$.

Results

The phosphorus and water (P and W) levels significantly affected the total plant dry mass ($p < 0.001$). The growth of the inbreds Norma B, 444 A and 444 B was poor compared to that of Norma A, and the hybrids Norma and Mv 444.

Though there was no great difference between the maximum shoot dry mass of Norma and the inbred Norma A, the latter had the highest shoot dry mass (SDM = 30.3 g shoot dry mass per plant at the P3M level, corresponding to SDM = 100% in Fig. 1).

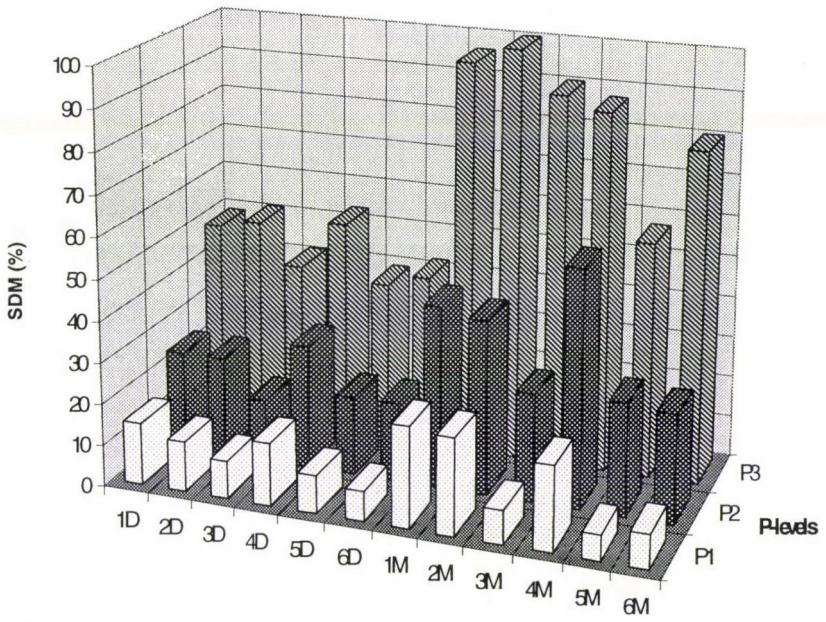

Fig. 1. Relative shoot dry mass of the genotypes. 1: Norma, 2: Norma A, 3: Norma B, 4: Mv 444, 5: 444 A, 6: 444 B. D: dry, M: moist soil conditions

In Table 1 some morphological and growth parameters are presented: root to shoot ratio (RWR, root dry weight per shoot dry weight, g/g), specific root length (SRL, root length per root dry weight, $m \cdot g^{-1}$), specific leaf area (SLA, leaf area per leaf dry weight, $m^2 \cdot kg^{-1}$), phosphorus taken up by unit root surface area (P/RA, shoot phosphorus content per root surface area, $mg \cdot cm^{-2}$) and root length density (Lv, root length per unit soil volume, $cm \cdot cm^{-3}$).

The estimation of the root to shoot ratio (RWR) is one way of quantifying the carbohydrate investment into above- and belowground plant parts, the photosynthetic area and the nutrient-acquiring root system. In general, cultivars or species with an extensive root development (high root : shoot ratio) may give higher yields under poor conditions, while cultivars with fewer roots may have higher yield potential, which can be obtained with a continuously high nutrient supply. Genotypes which respond quickly to a change in water availability with a change in the allocation of dry matter between roots and shoots probably perform better in environments where a small decrease in water availability is the start of a longer dry period, as happened in the present experiment. In environments where the water supply is never limited, a less responsive, more conservative genotype behaviour may give higher aboveground production.

Table 1
Morphological characteristics of the genotypes under different soil moisture and P supply conditions

Genotype	P added ($mg \cdot kg^{-1}$)	PWR ($g \cdot g^{-1}$)		SRL (mg^{-1})		SLA ($m^2 \cdot kg^{1}$)		P/RA ($mg \cdot cm^{-2}$)		Lv ($cm \cdot cm^{-3}$)	
		Dry	Moist	Dry	Moist	Dry	Moist	Dry	Moist	Dry	Moist
Norma	0	0.60	0.63	27.5	19.8	30.5	29.1	6.1	4.1	1.49	1.86
	25	0.86	0.66	35.4	25.8	20.2	25.9	2.8	5.9	4.63	4.63
	250	0.54	0.54	26.8	22.1	21.9	19.5	24.5	10.5	4.42	7.16
Norma A	0	1.31	0.69	33.8	38.5	32.6	30.1	2.6	6.2	3.25	3.93
	25	0.96	0.89	34.1	23.3	22.9	28.7	7.1	5.6	4.92	5.52
	250	0.58	0.72	24.3	16.4	25.2	20.6	11.6	25.4	4.49	7.49
Norma B	0	0.58	0.47	19.4	17.6	29.9	29.9	6.7	7.6	0.64	0.43
	25	0.51	0.51	36.1	14.0	29.5	30.0	4.71	12.9	1.71	1.16
	250	0.60	0.44	13.8	11.4	24.3	18.5	16.8	31.2	2.14	2.85
Mv 444	0	1.27	0.88	27.3	20.1	27.0	27.7	2.7	5.9	3.29	2.22
	25	0.92	0.78	31.4	19.9	21.4	22.4	2.75	6.0	4.47	5.58
	250	0.79	0.62	18.7	16.5	18.4	20.7	11.0	13.5	4.99	5.53
444 A	0	0.75	0.84	13.6	19.2	27.0	26.1	9.2	7.5	0.56	0.49
	25	0.58	0.38	23.6	13.2	21.4	25.5	5.2	26.9	2.40	0.73
	250	0.51	0.47	11.7	10.2	21.9	23.2	36.0	34.3	1.28	1.01
444 B	0	0.66	0.65	22.1	40.3	32.1	44.8	6.1	7.6	0.62	1.25
	25	0.66	0.52	12.8	29.2	22.9	28.9	11.9	7.9	1.02	2.51
	250	0.76	0.42	10.9	22.0	18.9	19.4	13.6	17.4	2.17	4.74

There is a functional equilibrium between root and shoot growth (de Willigen and van Noordwijk, 1987). The growth of leaf and root surface area as interfaces with the above- and belowground environment is governed by the plant genome and is constantly influenced by the environment of the plant.

A much higher proportion of the total plant was developed as roots in the inbred Norma A and the hybrid Mv 444 than in the other genotypes under phosphorus stress. For Norma A this was associated with a longer, finer root system and much lower phosphate influx, reflected in the SRL (specific root length) and P/RA (shoot P content per root surface area) values, respectively. A slight improvement in the soil phosphorus supply (P2 level) induced fine root growth (root growth at low carbon cost) as shown by the SRL values in Table 1. Under conditions of optimum water supply, SRL decreases with increasing phosphorus availability for all the genotypes except Norma, showing a general tendency towards the growth of thicker roots.

The attainment of high leaf area per unit leaf mass (SLA), i.e. the production of greater leaf area with less biomass, is considered as an adaptive growth strategy (Dijkstra et al., 1989). SLA was significantly higher in the P1 treatment than under P-fertilised conditions. The "mass density" of the leaves increased with increasing phosphorus supply under both dry and moist conditions. SLA was characteristic for all the genotypes, but the highest plasticity in response to the P supply was shown by the inbred 444 B.

Among the genotypes having long roots and good performance, the hybrid Norma, which had shorter, thicker roots, showed the highest shoot P uptake per unit root surface area under water- and phosphorus-deficient conditions.

For the uptake of higher amounts of available soil phosphorus and water, higher root length densities (Lv, $cm \cdot cm^{-3}$) are required. At optimum root length density the phosphorus and water acquired by the root system just equal the cost of root growth to the plant. Two groups can be distinguished as regards Lv for the six genotypes studied: (1) genotypes with high root length densities: Norma, Norma A and Mv 444, and (2) genotypes with low root length densities: Norma B, 444 A and 444 B. The latter group contains genotypes with much poorer growth under water- and P-deficient conditions than those in the first group. Root length density (Lv) proved to be highly sensitive to the soil phosphorus supply, especially in moist soil, where the slight increase in soil P in the P2 treatment markedly increased the root length density for all genotypes (see Table 1).

The SDM values reflect a significant effect of the P, W and P × W treatments except in the case of the inbred 444 A for the W and P × W treatments. RWR shows a P effect for Norma, Norma A and Mv 444. SRL indicates both P and W effects for all genotypes. SLA is a strong indicator of the P effect.

Table 2
MANOVA table for morphological characteristics at various P supplies (3 levels) and water supplies (2 levels)

Genotype	Effect	SDM	RWR	SRL	SLA
Norma	P	***	*	*	***
	W	***	n.s.	**	n.s.
	P × W	***	n.s.	n.s.	*
Norma A	P	***	*	*	***
	W	***	n.s.	n.s.	n.s.
	P × W	**	**	n.s.	**
Norma B	P	***	n.s.	*	***
	W	***	*	*	*
	P × W	***	n.s.	*	n.s.
Mv 444	P	***	***	*	***
	W	***	**	**	n.s.
	P × W	***	n.s.	n.s.	n.s.
444 A	P	***	n.s.	**	n.s.
	W	n.s.	n.s.	**	n.s.
	P × W	n.s.	n.s.	**	n.s.
444 B	P	***	n.s.	**	***
	W	***	*	***	n.s.
	P × W	**	n.s.	n.s.	n.s.

P = phosphorus supply, W = water supply. Levels of significance * = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$, n.s. = not significant

Water use efficiency (WUE kg dry matter kg^{-1} total water use, Veneklaas and Peacock, 1994) increased to a greater extent with the P supply in water-stressed than in non-stressed plants (Fig. 2a and b, respectively).

WUE was positively correlated with root length density ($R^2 = 0.67$ for all treatments). The hybrids proved to be efficient in water use, especially during water and phosphorus deficiency stress. The hybrid Norma was superior to the other genotypes in tolerating water and P deficiency stress, as demonstrated by estimating stress factors on the RGR (relative growth rate) basis for the Norma group (Table 3).

Stress factors are suitable parameters for comparing different responses to stress in various treatments for the genotypes. In the sub-optimum range there is a strong relationship between the water and phosphorus supplies and the shoot growth rate, which is why the stress factors have been calculated on a relative growth rate basis. The parental lines were much more sensitive to water and P stress (80–90% reduction) than the hybrid Norma (30-40 % reduction in RGR).

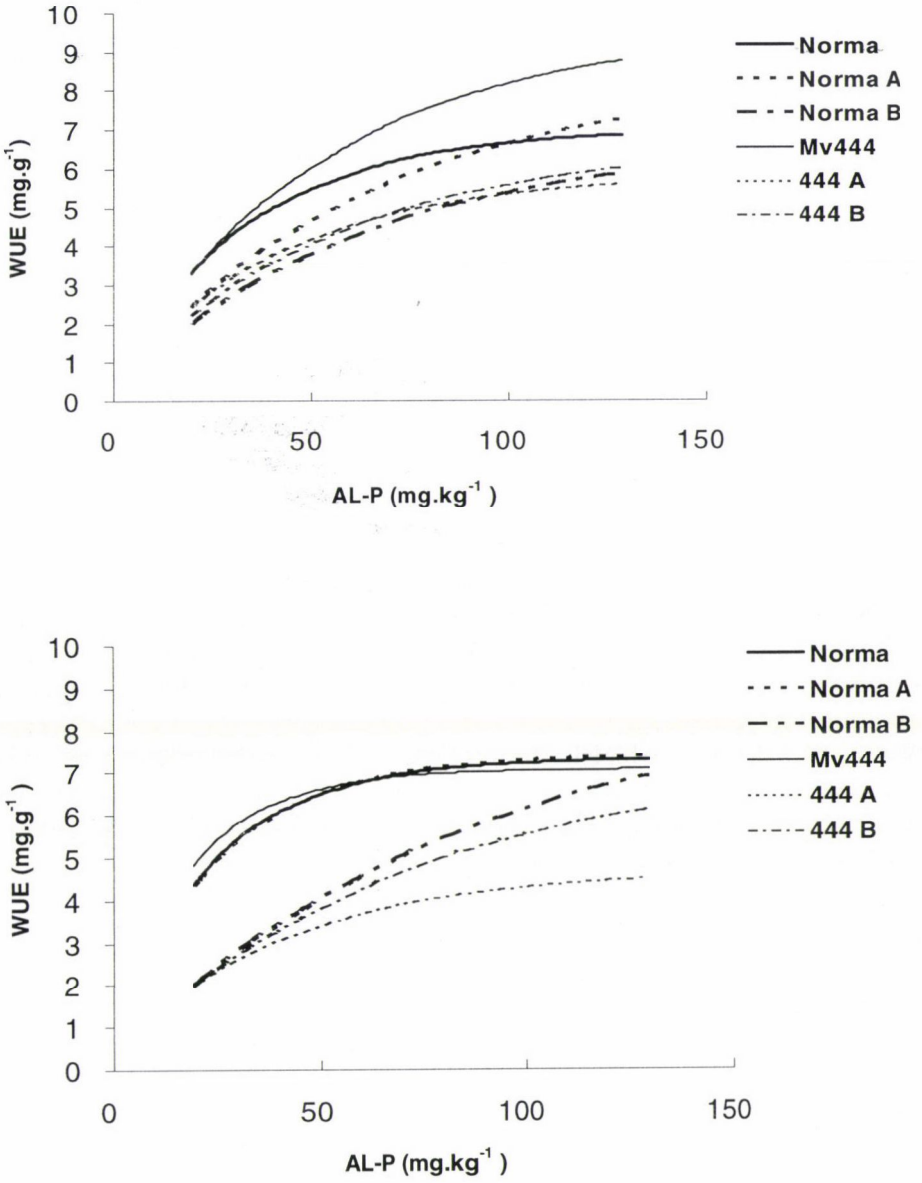

Fig 2. Effect of phosphorus supply on the water use efficiency (WUE) of the maize genotypes under dry (above) and moist (below) conditions

Table 3
Stress factors for the genotypes in the "Norma group"

Genotypes	Added P	Stress factors	
	($mg \cdot kg^{-1}$)	Under dry conditions	Under moist conditions
Norma	0	42	31
	25	55	59
	250	51	0
Norma A	0	84	89
	25	71	74
	250	50	0
Norma B	0	78	70
	25	77	20
	250	32	0

Conclusions

The plasticity in RWR due to the P level differed markedly, from Norma and Norma B with relatively stable RWR to Norma A and Mv 444.

A comparison of the genotypes showed that the partitioning of dry matter to the roots or a rapidly developing and finely divided root system associated with the production of greater leaf area with less biomass (the adaptation strategy adopted by the inbred Norma A) was less successful under conditions of severe drought and P stress than the greater uptake efficiency achieved by maintaining a higher influx and intensity of translocation and a higher utilisation of nutrients in metabolism and growth in Norma. The hybrids proved to be more efficient in water use, especially during water and phosphorus deficiency stress. In respect of stress tolerance, the hybrid Norma was superior to its parental lines, as reflected in stress factors estimated on a RGR basis (31–42 for Norma, 75–85 for Norma A and Norma B).

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YIELD AND YIELD COMPONENTS RESPONSE OF WHEAT (*TRITICUM AESTIVUM* L.) GENOTYPES GROWN UNDER DIFFERENT SOIL WATER DEFICIT CONDITIONS

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Compared with the control, water deficits reduced the yield and yield components in all the eight genotypes tested. However, genotypes Chakwal-86, DS-4 and Barani-83 had comparatively higher yield and yield components than the other genotypes. There was a positive correlation between yield and 1000 grain weight. The maximum reduction in all parameters was under terminal drought. The difference between pre- and postanthesis drought was non-significant, particularly for grain yield.

Key words: water deficit, grain yield, yield components, *Triticum aestivum* L.

Introduction

Wheat (*Triticum aestivum* L.) is a principal cereal crop in Pakistan. The average yield of wheat is fairly low due to various factors, including environmental stresses (Boyer, 1982), one of which is soil water deficit. Soil water deficit not only affects the morphology of the plants but also severely modifies the metabolism. The extent of this modification depends upon the variety/cultivar and the duration and intensity of the stress. In Pakistan, water is an important limiting factor, especially in rainfed areas which constitute nearly 1/3 of the total wheat planting acreage (Ashraf et al., 1994). The areas near the tail-ends of canals also face the problem of water shortage at various growth stages.

Grain yield is the ultimate product of many physiological processes occurring throughout the development of the plant till it dies. In *Gramineae* these include the number of flowers, the growth rate of the inflorescence, the number of grains, and the duration of grain filling and seed maturity. These processes are in turn integrated with leaf growth and its senescence. These processes form a balanced system of source and sink for assimilates and nutrients. All of these processes have been shown to be affected by water stress (Boyer and McPherson, 1977; Ashraf et al., 1994).

Materials and methods

An experiment was conducted in lysimeters with eight wheat genotypes at the Atomic Energy Agricultural Research Centre, Tando Jam, Pakistan during 1993–94. Each lysimeter measured 9 m^2 (3 m × 3 m) and was 1 m in depth. The lysimeters were separated by 15 cm thick cement walls which acted as a buffer zone on each side to prevent seepage. Prior to planting, the tanks were levelled to ensure an even distribution of water. The soil samples were analysed and it

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was found that they were deficient in nitrogen, organic matter and available phosphorus. Therefore, nitrogen and phosphorus were supplied as urea (70 kg N/ha) and diammonium phosphate (DAP) (35 kg P_2O_5/ha). The fertilizers were broadcast and mixed immediately prior to sowing. The seeds of the eight genotypes were hand drilled. Each genotype was allotted three rows of 80 cm in each tank. The row to row distance was 0.25 m and the plant to plant distance 7 cm. The crop was grown to maturity.

Seventy-five mm irrigation water was applied through a water meter as and when required. The following stress treatments were imposed to simulate the type of drought stress experienced and expected to occur in rainfed conditions as well as at the tail-end of canals.

Treatments:

Terminal drought:	No irrigation during crop growth, except the initial soaking dose (1 irrigation).
Postanthesis drought:	Irrigation applied for soaking, at tillering and at the initiation of heading (3 irrigations).
Preanthesis drought:	Irrigation applied for soaking, and at the anthesis and grain filling stages (3 irrigations).
Control:	Normal irrigation as recommended for wheat (irrigation applied for soaking, and at the tillering, heading, anthesis and grain filling stages -5 irrigations).

All lysimeters were protected from rain with a manually operated shelter equipped with a movable sheet of transparent plastic. The tanks were hand weeded and hoed whenever necessary. After harvesting, the yield and yield components were recorded and yield per hectare was calculated. The data were subjected to analysis of variance to determine the significance of differences between the treatments and/or genotypes. The differences were compared by Duncan's Multiple Range Test (DMRT) at the 5% probability level (Steel and Torrie, 1980).

Results

Water stress treatments reduced the number of grains per ear (Table 1). The highest reduction was observed under terminal drought (37.29%), followed by post- (22.57%) and preanthesis drought (18.54%). Barani-83, Chakwal-86 and DS-4 had a higher number of grains per ear after terminal, pre- and postanthesis drought than the other genotypes.

Significant differences between the treatments were observed for thousand grain weight (Table 2). The highest weight was noted under normal irrigation (42.38 g), followed by pre- (32.28 g), postanthesis (24.09 g) and terminal drought (19.38 g). Under terminal drought the highest thousand grain weight was recorded in DS-4 (25.21 g), followed by Barani-83 (24.38 g) and Chakwal-86 (23.95 g). The differences between post- and preanthesis were insignificant.

Grain yield was reduced under all drought treatments to various extents (Table 3). The maximum reduction was recorded under terminal drought (60%), but the reduction under post- and preanthesis drought was similar (43%). Under preanthesis drought the maximum yield was recorded in DS-4 and the minimum in DS-17 and Pavon. A similar trend was noted under postanthesis drought. Under terminal drought DS-4 and Chakwal-86 yielded maximum, while the lowest grain yield was recorded for DS-17.

Table 1
Effect of water stress on number of grains per ear in different wheat genotypes

Genotypes	Treatments			Control	Mean
	Terminal drought	Postanthesis drought	Preanthesis drought		
	D*	C	B	A	
Barani-83	44.50a	52.44a	56.56a	60.00b	53.38a*
	(26)**	(13)	(6)		
	C	B	B	A	
Chakwal-86	46.24a	54.69a	56.70a	64.00a	55.41a
	(28)	(14)	(11)		
	C	B	B	A	
DS-4	47.26a	54.22a	55.70a	61.80b	54.74a
	(23)	(12)	(10)		
	C	B	B	A	
DS-17	28.65bc	39.60c	41.00d	61.80b	42.76c
	(54)	(36)	(34)		
	C	B	B	A	
Pavon	29.02bc	38.74dc	41.25d	60.20b	42.30c
	(52)	(36)	(31)		
	C	B	B	A	
LU-26S	32.80b	45.63b	47.42c	64.60a	47.61b
	(49)	(29)	(27)		
	C	B	B	A	
Sarsabz	30.60b	42.06c	43.90d	64.40a	45.24bc
	(52)	(35)	(32)		
	D	C	B	A	
C-228	45.20a	48.00ab	52.60b	56.21c	50.50b
	(20)	(15)	(6)		
	D	C	B	A	
Mean	38.03	46.92	49.39	60.63	
	(37)	(23)	(18)		

*Means in the same column and same row sharing the same letter did not differ significantly according to Duncan's Multiple Range Test at the 5% level.
**Values in parentheses show percentage reduction from control.

Discussion

Grain yield depends on the number of fertile tillers surviving up to maturity, spike length, fertile spikelets, seed set per ear and grain size (thousand grain weight). In wheat, water deficit reduced the number of tillers initiated and surviving up to maturity, and the number of seeds set per tiller (Ashraf et al., 1987; 1989). In the present study both the number of tillers per plant and the number of grains per ear were reduced by water stress (data not shown in the tables), depending on the timing of the drought. In the terminal drought the reduction in tillers per plant was 22%, while under postanthesis drought it was

15%. Surprisingly, under preanthesis drought there was no significant difference. From the above results it seems that the postanthesis stage was the most sensitive stage in the genotypes tested.

Nachit (1984) and Ashraf et al. (1987; 1989) found high correlations between the number of grains per ear, 1000 grain weight and the grain yield. In the present study 1000 grain weight was influenced by water stress applied at different growth stages and was significantly correlated with grain yield (r = 0.747*) (Fig. 1). The grains were shrivelled under stress conditions and the degree of shrivelledness depended on the genotype. Biryukov and Lyashok (1983) found that drought, at different stages, affected the number of grains per ear and 1000 grain weight. Genotypes with a higher number of grains per ear and 1000 grain weight produced more yield. However, they suggested that this was not valid under all conditions of drought as they did not find any correlation for drought at the tillering stage. Rusalka, a tolerant genotype, showed only a 9% decrease in 1000 grain weight.

On the other hand, Talukudar et al. (1989) and Khan et al. (1993) showed that the grain yield reduction caused by water stress was mainly due to a reduction in 1000 grain weight. The genotypes Chakwal-86, Barani-83 and DS-4 had higher 1000 grain weights compared to the other genotypes used in this study (Table 2), which suggests that 1000 grain weight does play a role in increasing yield under drought.

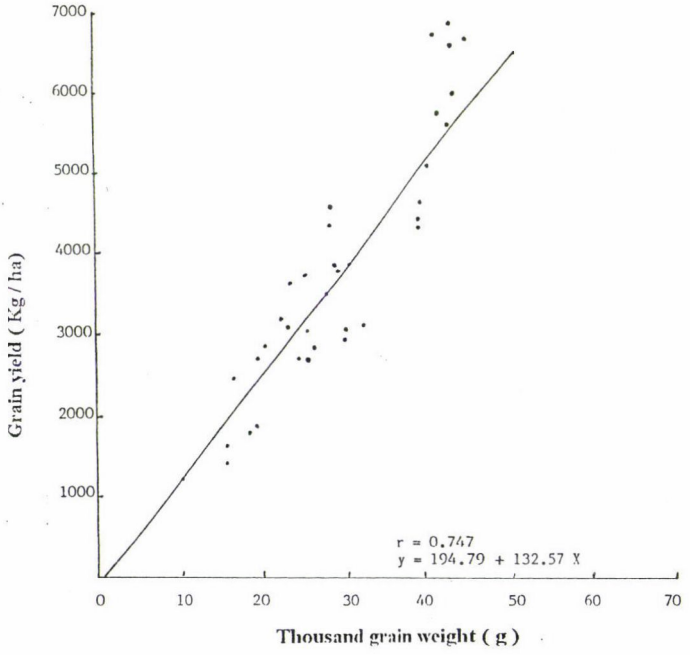

Fig. 1. Relationship between thousand grain weight and grain yield

The sensitivity of grain yield to drought stress other than terminal drought depends upon the severity of the stress and the stage when it is applied. Postanthesis stress caused a greater reduction in grain yield than preanthesis stress (Talukuder et al., 1987). In the present study, the differences between pre- and postanthesis were non-significant. The apparent differences in our findings are perhaps due to conditions other than water deficits. However, when the average of all the genotypes was compared, it was observed that the grain yield was better after postanthesis drought. In the present study six of the seven genotypes tested responded similarly to pre- and postanthesis drought (Table 3). It is obvious that factors other than water must have played a role in the discrepancy found. From the above comparison it would be reasonable to conclude that as far as grain yield is concerned there is no difference between pre- and postanthesis drought.

Table 2
Effect of water stress on thousand grain weight (g) of different wheat genotypes

Genotypes	Treatments			Control	Mean
	Terminal drought	Postanthesis drought	Preanthesis drought		
	D*	C	B	A	
Barani-83	24.38a	27.84a	39.25a	43.51b	33.75a*
	(44)**	(36)	(10)		
	D	C	B	A	
Chakwal-86	23.95a	27.64a	38.97a	43.07b	33.41a
	(44)	(36)	(10)		
	D	C	B	A	
DS-4	25.21a	27.72a	38.80a	40.51c	33.06a
	(38)	(32)	(14)		
	D	C	B	A	
DS-17	15.25d)	19.25d	23.72e	41.70cb	24.98d
	(63	(54)	(43)		
	D	C	B	A	
Pavon	15.39d	20.25d	25.89d	42.92b	26.11c
	(64)	(53)	(40)		
	D	C	B	A	
LU-26S	18.72b	24.55b	32.12b	45.09a	30.12b
	(58)	(46)	(29)		
	D	C	B	A	
Sarsabz	18.92b	23.25b	30.22c	43.01b	28.85b
	(56)	(46)	(30)		
	D	C	B	A	
C-228	17.22c	22.26bc	29.30c	39.22cd	27.00c
	(56)	(43)	(25)		
	D	C	B	A	
Mean	19.88	24.09	32.28	42.38	
	(53)	(43)	(24)		

*Means in the same column and same row sharing the same letter did not differ significantly according to Duncan's Multiple Range Test at the 5% level.
**Values in parentheses show percentage reduction from control.

Table 3
Grain yield (kg ha^{-1}) of eight wheat genotypes grown under different water regimes

Genotypes	Treatments			Control	Mean
	Terminal drought	Postanthesis drought	Preanthesis drought		
	C*	B	B	A	
Barani-83	2774b	3844c	3937c	6072c	4157c*
	(54)**	(37)	(35)		
	C	B	B	A	
Chakwal-86	3642a	4434b	4352b (	6586b	4754b
	(45)	(33)	34)		
	C	B	B	A	
DS-4	3745a	4635a	4596a	6742a	4929a
	(44)	(3l)	(32)		
	C	B	B	A	
DS-17	1442f	2790e	2760f	5745d	3184e
	(75)	(51)	(52)		
	C	B	B	A	
Pavon	1690e	2925e	2874f	5642b	3283e
	(70)	(48)	(49)		
	C	B	B	A	
LU-26S	1820d	3094d	3046e	6671b	3658d
	(72)	(54)	(54)		
	C	B	B	A	
Sarsabz	1921d	3046d	3126d	6890a	3746
	(72)	(56)	(55)		
	C	B	B	A	
C-228	2477c	2986d	3243d	4342e	3262e
	(43)	(31)	(25)		
	C	B	B	A	
Mean	2473	3469	3491	6086	

*Means in the same column and same row sharing the same letter did not differ significantly according to Duncan's New Multiple Range Test at the 5% level.
**Values in parentheses show percentage reduction from control.

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STUDIES ON THE STANDARDIZATION OF NURSERY METHOD, SEEDLING AGE AND PLANT DENSITY FOR LOWLAND RICE ESTABLISHED BY THE SEEDLING BROADCASTING METHOD

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A field experiment was conducted during the 1994–1995 wet and dry seasons to standardize the nursery method, seedling age and plant density for the seedling broadcast method of planting lowland rice. The yield of rice achieved by the seedling broadcast method (5.28 and 6.26 t ha^{-1} in the wet and dry seasons, respectively) was comparable with the traditional random planting method. Although the line planting method enhanced the yield to 5.80 and 6.65 t ha^{-1} in the wet and dry seasons, respectively, the seedling throwing method is nevertheless more advantageous because of the lower labour requirements (50.7% of that of line planting and 76.7% of that of random planting) and the relative ease with which the seedlings are thrown. As such, the advantage of the seedling broadcasting method resulted in a higher B:C ratio (2.59 and 3.02 for the wet and dry seasons) compared to the line planting method (2.49 and 2.99 in the wet and dry seasons, respectively). Studies on the evaluation of component technologies to enhance the yield of seedling broadcast rice revealed that irrespective of the nursery method, the use of older seedlings (35 days old) at 30% greater plant density maximised the yield to 5.73 t ha^{-1} during the wet season, which is on par with that of line planting (5.86 t ha^{-1}). In the dry season, the broadcasting of 35-day-old seedlings from a semidry nursery at normal plant density itself produced a higher yield (6.67 t ha^{-1}), which is marginally more than that of line-planted rice (6.65 t ha^{-1}).

Key words: rice establishment, seedling broadcasting, nursery method, plant density

Introduction

The method of stand establishment is one of the cultural practices that influences the performance of rice through its effect on growth and development (Mian and Ahsan, 1969; Nair et al., 1973). Transplanting (in line or randomly), the most widely practised rice planting method, is often not done at the right time due to the increased demand and scarcity of labour (Varughese et al., 1993). The need for a satisfactory alternative to the transplanting of rice, in the context of the scarcity and increasing cost of human labour and the desire to reduce the drudgery of the women, has been very much felt and attempts were made in this direction. Though mechanical rice transplanting has been developed, it is not suitable for small, fragmented holdings or for the socio-economic conditions of rice farmers in India. In South East Asian countries, a method of rice crop establishment by simply throwing the rice seedlings into the

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puddle has been developed to achieve better management and yield in rice cultivation (Matsushima, 1979). Nursery management is a very important component technology for obtaining healthy, vigorous seedlings. Seedling age at transplanting also plays a crucial role in realising the potential yield and behaves differently on the basis of the duration of the variety. Matsushima (1979) recommended a longer nursery period for the seedling broadcast method of planting rice. Though works on population density for line transplanting have been fairly exhaustive, there is a need to standardize the population density for the seedling broadcast method of transplanting.

The objectives of this study were to evaluate the suitability and success of the seedling broadcast method of rice planting and to optimise the nursery method, seedling age and plant density for this planting method to obtain a yield comparable with that of the line and random planting methods while involving lower labour requirements and greater ease of operation.

Materials and methods

The experiments were conducted on the wet lands of Tamil Nadu Agricultural University, Coimbatore during the wet season (October 1994 to February 1995) and the dry season (June to September 1995). The soil texture was clay loam. The available nutrient status of the soil was characterised by low nitrogen, medium phosphorus and high potassium contents.

The experiment was laid out in a factorial randomised block design with three replications. The twelve treatment combinations involved three types of seedlings from two nursery methods (normal wet nursery seedlings, wet nursery seedlings dipped in 1:1 clay and cowdung slurry, and seedlings from a semidry nursery), two seedling ages (25 and 35 days old) and two plant densities (normal and 30% greater) for the seedling broadcast method of planting. The performance of the seedling broadcast planted crop under the above treatment combinations was compared with line and random planted crops that were grown with the recommended package of production practices.

The field was puddled and uniformly levelled. In the line planting method, 25-day-old seedlings from a wet nursery were transplanted at a spacing of 20cm × 10 cm in the wet season and
15 cm × 10 cm in the dry season. Random planting was done by transplanting 25-day-old seedlings from a wet nursery without adopting any definite plant geometry and density, as done by farmers.

Twenty-five- and thirty-five-day-old seedlings from wet and semidry nurseries were used for the seedling throwing method. Two or three seedlings were separated as in normal transplanting and were broadcast at random in the respective plots by hand from a standing position without using force. The population in the normal plant density treatment in the broadcast planting method was maintained equal to the pre-decided population in the line planting treatment, calculated according to the plant spacing adopted in the respective seasons. In the treatments involving 30% greater plant density, 30% more seedlings were broadcast. The labour requirements for each method of planting were recorded and given in woman days ha^{-1}. The crop yield was determined from the net plot area of 6 m^{-2} and expressed in t ha^{-1} at 14% moisture.

Results and discussion

Effect of planting methods on yield parameters and yield

The response of the individual yield components varied significantly with the different planting methods (Table 1). The seedling broadcast and line planting methods of crop establishment produced equal numbers of panicles m^{-2} (523 and 527, respectively), while the panicle production was significantly lower with random planting (491) in the dry season. This parameter was not much influenced by planting methods in the wet season. Line planting resulted in substantially higher filled grains $panicle^{-1}$ values (109.7 and 93.7 in the wet and dry seasons, respectively) compared to the other two planting methods. Higher spikelet sterility in the seedling throwing method of planting was reported by Shekhar and Singh (1991). The seedling broadcast method of planting is superior to the random planting method, as is evident from the yield parameters studied.

Table 1
Effect of treatments on panicle and grain production of wet and dry season rice

Treatments	Wet season			Dry season		
	Panicles m^{-2}	Filled grains $panicle^{-1}$	Unfilled grains $panicle^{-1}$ (%)	Panicles m^{-2}	Filled grains $panicle^{-1}$	Unfilled grains $panicle^{-1}$ (%)
Establishment methods						
Seedling broadcast planting	477	83.7	17.3	523	78.8	22.8
Line planting	507	109.7	10.1	527	93.7	16.4
Random planting	461	80.4	15.6	491	76.5	22.6
CD (P=0.05)	NS	14.8	NS	25.7	5.8	NS
Treatments for seedling broadcast planted rice						
Nursery methods						
Wet nursery	474	82.5	18.3	517	77.0	24.1
Wet nursery-slurry dip	482	82.0	16.8	521	78.1	23.2
Semidry nursery	475	86.6	16.7	531	81.2	23.1
CD (P=0.05)	NS	NS	NS	NS	3.9	NS
Seedling age						
25 days	445	74.0	22.8	501	73.2	28.0
35 days	509	93.4	11.7	545	84.4	17.6
CD (P=0.05)	21.2	8.2	4.80	14.2	3.2	4.12
Plant density						
Normal	466	79.6	19.2	514	77.1	24.7
30% greater	488	87.8	15.3	532	80.4	20.9
CD (P=0.05)	21.2	8.2	NS	14.2	3.2	NS

The crop established by the seedling broadcast method produced grain yields which are on a par with random planting (5.28 and 6.26 t ha^{-1} in the wet and dry seasons, respectively). However, the highest grain yield (5.80 t ha^{-1} in the wet season and 6.65 t ha^{-1} in the dry season) was recorded for line planting. The increased nutrient uptake and better yield parameters recorded in the seedling broadcast and line planting methods were manifested in higher yields. A higher grain yield with the seedling broadcast method was earlier reported by Matsushima (1979) and Varughese et al. (1993).

Effect of component techniques on the growth and yield of seedlings of broadcast-planted rice

The panicle number, filled grains $panicle^{-1}$ and test weight of the grain were not significantly influenced by the nursery methods adopted, though a higher number of panicles was obtained with semidry nursery seedlings (531) in the dry season and with wet nursery seedlings dipped in slurry (482) in the wet season. The significantly higher number of filled grains $panicle^{-1}$ for the crop established with semidry nursery seedlings (81.2 compared to 77.0 with wet nursery seedlings in the dry season) might be due to a higher nutrient uptake and higher LAI.

The results of the study indicated a definite role of seedling age in the growth and yield of rice established by the seedling broadcast method. Older seedlings produced 14 and 8% more panicles m^{-2} over normal-aged seedlings in the wet and dry seasons, respectively. They also increased the filled grains $panicle^{-1}$ rate as a result of greater photosynthesising area and better nutrient uptake. Earlier, Matsushima (1979) also reported that the longer the seedlings were kept in the nursery, the better were the results obtained after broadcast planting. Increasing the plant density by 30% increased the number of panicles m^{-2} and the number of filled grains over normal plant density due to better vegetative growth parameters during both seasons. Normal plant density registered 4.5 and 3.3% less panicles m^{-2} than 30% greater plant density. The higher proportion of unproductive tillers at normal planting density resulted in a lower number of panicles m^{-2}.

Though the nursery methods did not influence the grain yield in the wet season, the use of seedlings from a semidry nursery or of seedlings from a wet nursery dipped in slurry substantially enhanced the grain yield to 6.36 and 6.28 t ha^{-1}, respectively, compared to 6.14 t ha^{-1} for normal wet nursery seedlings. The crop established using seedlings from a semidry nursery was observed to have better root development, higher leaf area index and dry matter production, more panicles m^{-2} and more filled grains $panicle^{-1}$, which resulted in a higher grain yield. All the observed yield parameters, namely spikelets $panicle^{-1}$, productive tillers m^{-2} and filled grains $panicle^{-1}$, were higher for the crop planted with older

seedlings and for treatments involving the use of 30% more seedlings during both seasons and the resultant higher yield might be the cumulative effect of all these increases. Varughese et al. (1993) also reported a higher yield with crops established by broadcasting the seedlings.

Effect of treatments on labour requirements and economics

The seedling broadcast method of planting is rated to be less labour-intensive and to involve less drudgery for the planters compared to the line planting and random planting methods. The method of seedling throwing required only 50.7% and 76.7% of the planting labour used for line and random planting, respectively. A similar reduction in the labour force was reported by Lal et al. (1986) and Mao Bi Jun (unpublished).

The lower grain yield obtained with the seedling broadcast method compared to line planting was counterbalanced by the lower costs of cultivation and it was found to record the highest B:C ratio of 2.59 and 3.02 in the wet and dry seasons, respectively (Table 2). The net income and B:C ratio recorded in the seedling broadcast method also exceeded those of random planting.

Table 2
Effect of treatments on grain yield and economic indices of wet and dry season rice

Treatments	Wet season			Dry season		
	Grain yield ($t\ ha^{-1}$)	Net returns (US $)	B : C ratio	Grain yield ($t\ ha^{-1}$)	Net returns (US $)	B : C ratio
Establishment methods						
Seedling broadcast planting	5.28	414.09	2.59	6.26	536.14	3.02
Line planting	5.80	446.71	2.49	6.65	554.97	2.88
Random planting	5.28	408.06	2.51	6.15	517.14	2.92
CD (P = 0.05)	0.26			0.17		
Treatments for seedling broadcast planted rice						
Nursery methods						
Wet nursery	5.25	413.91	2.59	6.14	523.31	2.99
Wet nursery-slurry dip	5.32	414.97	2.61	6.28	538.86	3.04
Semidry nursery	5.27	413.40	2.56	6.36	546.26	3.04
CD (P = 0.05)	NS			0.12		
Seedling age						
25 days	4.94	373.09	2.42	6.04	508.26	2.92
35 days	5.62	455.11	2.75	6.48	564.03	3.13
CD (P = 0.05)	0.15			0.10		
Plant density						
Normal	5.15	400.60	2.58	6.21	536.20	3.08
30% greater	5.41	427.60	2.60	6.32	536.11	2.97
CD (P=0.05)	0.15			0.10		

The crop established by broadcasting semidry nursery seedlings recorded a higher B:C ratio of 3.04 during the dry season than in the wet season (Table 2). The higher net income and B:C ratio recorded when broadcasting older seedlings was due to the higher grain yield. Though higher grain yields were obtained with 30% higher plant density during both seasons, in the dry season the normal planting density led to a higher net income (536.20$) and B:C ratio (3.08). In the wet season, 30% more seedlings were necessary to offset the loss in the establishment of the seedlings due to rain, but in the dry season, the establishment was good and the use of 30% more seedlings led to higher production costs.

The results of the two-season study suggest that the new method of broadcasting rice seedlings on to puddled soil is simple, practically feasible and less labour-intensive (Table 3). This practice requires careful water management for the first 7–10 days of the establishment stage. In the wet season, the use of 35-day-old seedlings at 30% greater density, irrespective of the nursery method, and in the dry season, seedlings of the same age from the semidry nursery broadcasted at normal plant density were found to be appropriate production technologies for obtaining higher yields and better economic indices when establishing the rice crop by broadcasting the seedlings rather than transplanting them.

Table 3

Planting labour requirement for different establishment methods (woman days · ha^{-1})

Establishment method	Wet season 1994–95	Dry season 1995	Saving over line planting (%)	
			Wet season	Dry season
Seedling broadcasting				
Normal plant density	35	37	56.3	51.3
30% greater plant density	43	43	46.3	43.4
Line planting	80	76	–	–
Random planting	52	51	35.0	32.9

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PLANT DENSITY AND YIELD RELATIONSHIPS OF INTERCROPPED MAIZE AND CASSAVA

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Plant population density is critical to the yield of field crops and appears to be more so when these crops are interplanted as intercrops. Different stands of two maize (*Zea mays* L.) varieties and two cassava (*Manihot esculenta* Cranz) varieties were established as intercrops in experiments at Fumesua and Kwadaso in Kumasi (60°43' N, 10°36' W) and at Pokuase, near Accra (50°36' N, 00°10' W), from 1986–1989. The objective was to investigate the yield/density relationships of maize and cassava when intercropped. The results indicated that there was a need to strike a balance between the respective densities of the components to obtain better yields. The results also showed that about 40,000 plants ha^{-1} maize density was the maximum plant density needed to intercrop maize with cassava. The maturity period of the variety will determine whether this should be dropped to a lower level. For cassava, the maximum should be about 20,000 plants ha^{-1} with similar suggestions to those recommended for intercropped maize.

Key words: resource-poor farmers, intercropping, plant population density, optimum density, asymptotic

Introduction

In the resource-poor farmer's context, the often cited disadvantages of intercropping, for example, impractical management in a situation of high-level mechanization, or where the components have different fertilizer, herbicide and pesticide requirements, are not relevant. Such farmers have never depended on modern, often complex and heavily mechanized agriculture.

The maize and cassava intercrop is the most prominent of the food crop associations in Ghana and since maize and cassava intercrop-farmers fall into the category mentioned above, for them the advantages of intercropping cited in the literature (Wrigley, 1969; Osiru, 1982) are valid. Various crop combinations with cassava have been studied. Some of these (Ezumah, 1990) were: cassava + beans; maize + cassava; cassava + plantain + cocoyam; cassava + peanuts and cassava + cowpeas. The combinations were such that a land equivalent ratio (LER) of at least 1.5 was common. When maize and cassava are intercropped, the resulting competition tends to be severe during the first four months, which is a relatively early period in the life of cassava. In fact, the fast-growing maize exploits the environment early and the slow-growing cassava exploits it at a later period after the maize harvest (Wilson and Lawson, 1980). Again, Wilson and Lawson (1980) have found that competition between maize and cassava occurs mainly during the period when maize makes its peak demand on

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resources, which cannot last more than four months. Although cassava is suppressed by maize during early growth, it recovers rapidly after the maize has been harvested. The yield of cassava may therefore be reasonably high, since tuberization occurs during the long post-competition period. Temporal compatibility would be expected in a crop combination such as intercropped maize and cassava, because maize is usually harvested at 120 days or less, while the duration of cassava extends to 360–550 days (Ezumah et al., 1990). Yet the cassava yield is often reduced by the short period during which maize interfered.

When two or more crops are interplanted such that most or part of their life cycles coincide, the factors determining the yield of the component crops will include the choice of variety and agronomic practises.

Work at Fumesua (GGDP, 1986; 1987) has shown that when a full-season maize was interplanted with a long-duration cassava, the reduction in yield due to their association was 12% for the maize grain yield and 27% for the dry cassava tuber yield. This suggested that maize was the dominant species in such a crop association. Under such circumstances, Willey (1979) suggested that a greater effort should be made to select suitable genotypes of the crops which form the dominated species because they might grow under low light intensity conditions due to the modification of their environment by the dominant crop, particularly through shading. Often cassava was dominated by maize populations of over 40,000 plants/ha, as reported for the humid tropical alfisol at IITA near Ibadan, Nigeria (Ezumah et al., 1990). The reduction in cassava tuber yield was frequently more pronounced if a spreading, highly vegetative maize type was used in the intercrop system. Cassava height and internode length were found to increase significantly as the maize population increased, while the leaf number per plant decreased 60 DAP (Ezumah et al., 1990). The authors also noted that at maize harvest, maize population effects on cassava were still observed in cassava height and internode length, which increased with an increasing maize population. The stem diameter, branches per plant and leaves per plant decreased significantly.

Work in Nigeria (Ezumah et al., 1986) suggested that maize selected for high yield as a monocrop may do quite well as an intercrop with cassava. The aim in such a selection in maize would be to select for characters which could reduce the competitive effects on the cassava component, and still give an acceptable yield of maize. Another study at IITA (Ezumah, 1990) indicated that the yield responses of sole-cropped maize could be used to accurately predict its responses when intercropped with cassava. However, the effects of maize varieties on the associated cassava yield varied with maize growth habits, especially in maize populations higher than 40,000 plants/ha.

This study dealt with the yield and plant density relationships of maize and cassava when intercropped.

Materials and methods

The experiment consisted of three trials, involving interplanted maize and cassava as intercrops, to estimate the relationships between the plant density and yield of the two components. The experiments were conducted at three locations in 1986–1989. There are two rainy seasons, major and minor, in these locations. The rains in the major season normally begin in March and end in July. There is a short dry spell in August and then the minor season rains begin and end in October or November. Fumesua and Kwadaso are two similar locations in Kumasi (60°43' N, 10°36' W) in the central forest belt of Ghana, while Pokuase is near Accra (50°36' N, 00°10' W) in the south coastal savanna area of Ghana. The varieties used were: 1) *Dorke*, a 90-day, early, white dent maize variety. It is open-pollinated and was developed in 1984 by the Crops Research Institute, Kumasi, Ghana; 2) *Dobidi*, a 120-day, full season white dent variety. This again is an open-pollinated variety developed at the Crops Research Institute, Kumasi, Ghana, in 1984; 3) Bosome Nsia, an early-maturing (8–9 months) but late-branching local cassava cultivar grown mainly in the coastal savannah areas of Ghana. It grows to a height of about 180 cm in 9 months. It probably originated from a local collection from Kpeve in the Volta Region of Ghana which was called 'Asram Asia' meaning a 'six month' variety; 4) Ankra, a long-season (12–18 months maturity) late-branching local cassava cultivar widely grown in the forest and transitional areas of Ghana. It was selected in 1933, according to Doku (1965), from an introduction from Mauritius. It grows to a height of about 280 cm in 12 months and is very susceptible to African cassava mosaic virus (ACMV).

The soils at the experimental sites generally support a wide variety of cereal crops, root and tuber crops and grain legumes. Continuous cultivation, with fertilizer application and exposure of the soil to the tropical weather, are known to have affected the properties of these soils. The continuous N fertilizer application on these soils has contributed to reducing the soil pH to below 6 and soil organic matter levels are very low. With the intensive cropping practised on these soils for over 10 years, N, P and some micronutrients may become limiting.

The description of the soils of the three locations where the trials were conducted is as follows:

1) Fumesua

The soil series at this location is the Asuansi series and has been classified as Ferric Acrisol (FAO/UNESCO legend) or Paleustult (USDA soil Taxonomy). The common slope gradients range from 2-6%. The top soil usually has 2 to 3 layers. The top layer, about 5 cm thick, is dark grey gritty loam to gritty clay loam. The subsoil contains mainly quartz gravel in a clay matrix. Mixed ironstone concretions and quartz gravel are contained in the clay matrix in some profiles. The soils are deep, porous, well drained and well aerated with a good tilth. Moisture retention is fairly good in the sub-soil but the upper horizons tend to dry out rapidly during prolonged dry spells. The plots used for the trials at this location had a previous history of continuous cultivation with maize and cassava and were fallowed for about 3 years.

2) Kwadaso

The soils of this location belong to the Kumasi series and the parent material of this soil, classified as Ferric Acrisol (FAO/UNESCO) or Paleustult (USDA), is weathered granite. The soil occurs on gentle to moderately steep upper slopes. The soil of the series generally has good physical conditions for plant growth. It is deep, porous, freely drained, well aerated and has good tilth. Moisture retention is fairly good in the sub-soil but the upper horizons tend to dry out rapidly during prolonged dry spells. The top soil has at least two or more layers with a total thickness of about 16–21 cm. The sub-soil consists of a red to yellowish red clay loam or clay containing quartz gravel and ironstone concretions. The plots cropped had a previous history of cultivation, especially with maize, for over 10 years.

3) Pokuase

The Pokuase soils belong to the Akroso series. The parent material of this soil, classified as Dystric Cambisol (FAO/UNESCO) or Dystrochrepts (USDA), is a colluvium derived from granite. It occurs on gradients of 2–6%. The solum is relatively free of concretions and gravel. The depth of non-concretionary transported material varies between 45-150 cm. The series has good physical conditions for plant growth and since the horizons are usually free of concretions and coarse gravel, they are better suited to mechanized cultivation. The series is generally low in plant nutrients. The sub-soil texture varies from loam through clay loam to clays. The site used for the experiments had supported continuous maize cultivation for over 10 years.

Cultural practises

At all locations where the trials were conducted, the land was prepared by disc ploughing and harrowing to obtain a smooth seed bed. Unbranched hardwood middle stems of cassava were obtained from a uniform bulking plot planted one year earlier. The cassava stem cuttings were approximately 20 cm long and were planted on the flat in rows spaced 1 m apart. Maize seed was protected from predators by Furadan 350 ST (carbofuran) at a rate of 30 ml commercial product in 15 ml water per kilogram of seed.

Row length was 8 m and plots measured 8 m × 8 m. In all the trials, cassava was planted in rows between two adjacent maize rows. Row width was 1 m such that two rows of cassava were spaced 1 m while two rows of maize were also spaced 1 m. The cassava row was 50 cm from a maize row and vice versa. For the intercrops, the interplanting of cassava into the maize was carried out at the same time as maize planting, except in situations where time did not permit this. In such cases, the cassava planting was completed within eight days of the planting of the maize. For the monocrops, the maize or cassava was planted at the same time as the intercropped maize and cassava, respectively, were planted.

The fertilizer applied to maize, unless otherwise stated, was equivalent to 90:38:38 (made up of 38:38:38 kg/ha $N:P_2O_5:K_2O$ of compound fertilizer at planting and 52 kg/ha N as sulphate of ammonia side-dressed at 5-6 WAP maize). All fertilizer applications were made by banding in furrows, 5 cm from the maize plants. Plant density, fertilizer rates and other husbandry practices for monocropped maize and cassava were those recommended for the varieties when sole-cropped.

In all the trials, the within-row spacings for maize and cassava were adjusted to give the desired plant population densities.

Weeds were controlled in each trial by a pre-emergent application of Primagram 500, a herbicide made up of a combination of 250 g/l metolachlor (CIBA-GEIGY), 235 g/l atrazine (DU-PONT) and 10 g/l atrazine-related compounds, at a rate of 2.0 kg a.i./ha. [Metolachlor = 2-chloro-N-(2-ethyl-6-methyl-phenyl)-N-(2-methoxy-1-methylethyl) acetamide. Atrazine = 2-chloro-4-(ethylamino)-6-(isopropylamino)-s-triazine.]

Supplementary handweeding was done in all the trials where necessary to control weed regrowth after the herbicide application.

The maize harvesting period was between 90 and 105 DAP for 'Dorke' and between 120 and 130 DAP for 'Dobidi'. The cassava harvesting date, unless specified, was not earlier than 9 months and not later than 12 months for 'Bosome Nsia'. For 'Ankra' harvesting was not earlier than 12 months. The grain yield of maize was corrected to 15% moisture, while the storage root yield of cassava was either given on a fresh weight basis or on a dry weight basis (air-forced oven at 70°C for 2–3 days).

Trial 1

In Trial 1 at Fumesua and Pokuase, 1986, the design was a random complete block with 4 replicates. The treatment combinations for the intercrop were as shown in Table 1.

The planting dates for maize were: 9 Apr. at Fumesua, 1986; 30 May 1986 at Pokuase. Planting dates for cassava were: 15 Apr. 1986 at Fumesua and 3 Jun. 1986 at Pokuase.

Table 1

Treatment combinations using 'Dobidi' and 'Ankra' as the maize and cassava varieties respectively in Trial 1 at Fumesua and Pokuase, 1986

Maize density (plants/ha)	WRS*(adjusted) (cm)	No. of plants /hill	Cassava density plants/ha	WRS* (adjusted) (cm)
70,000	28.6	2	3,000	333.3
60,000	33.3	2	6,000	166.7
50,000	40.0	2	9,000	111.1
40,000	50.0	2	12,000	83.3
30,000	33.3	2	15,000	66.7
20,000	50.0	1	18,000	55.6
10,000	100.0	1	21,000	47.6

*WRS: within-row spacing

Trial 2

In Trial 2, in 1987, the design was a split plot. The main plot for the intercrops was either 'Dorke' + 'Bosome Nsia' or 'Dobidi' + 'Ankra'. The choice of such pairings was based on the findings of 1986 which gave a clear indication as to the combinations essential to minimize intercrop competition. The sub-plots for the intercrops were 7 density levels at Kwadaso (Table 2), while at Pokuase, the sub-plots were 6 density levels (10,000 to 60,000 plants/ha in 1987; Table 2).

The planting dates at Pokuase were: 24 Apr. 1987 for both the maize and the cassava. At Kwadaso, the treatments in Trial 2 in 1987 were similar to those at Pokuase except that the 70,000 and 6,000 plants/ha densities for maize and cassava respectively were not included. The planting date was 9 Apr. 1987 for both the maize and the cassava.

Table 2

Treatment combinations: 'Dobidi' and 'Ankra' , the maize and cassava varieties respectively at Kwadaso; 'Dorke' and 'Bosome Nsia', respectively at Pokuase, 1987

Maize density (plants/ha)	WRS*(adjusted) (cm)	No. of plants /hill	Cassava density plants/ha	WRS* (adjusted) (cm)
70,000	28.6	2	6,000	166.7
60,000	33.3	2	9,000	111.1
50,000	40.0	2	12,000	83.3
40,000	50.0	2	15,000	66.7
30,000	33.3	2	18,000	55.6
20,000	50.0	1	21,000	47.6
10,000	100.0	1	24,000	41.7

*WRS: within-row spacing

Trial 3

The treatments and design of Trial 3 at Fumesua and Pokuase in 1989 involved a combination of 'Dobidı' and 'Ankra' at Fumesua and 'Dorke' and 'Bosome Nsia' at Pokuase. The factors under study were 5 levels of maize density: 20, 40, 50, 60 and 90 thousand plants/ha. This was interplanted with cassava at 10,000 plants/ha and 3 levels of maize fertilization, i.e. 0, 45:19:19 (made up of 19:19:19 kg/ha N:P_2O_5:K_2O as compound fertilizer at planting and 26 kg/ha N as sulphate of ammonia side-dressed at 5–6 WAP maize), and 90:38:38 kg/ha (made up of 38:38:38 kg/ha N: P_2O_5:K_2O of compound fertilizer at planting and 52 kg/ha N as sulphate of

ammonia side-dressed at 5–6 WAP maize). Planting dates were: 1) intercropped maize, 5 Apr. 1989 and 2) intercropped cassava, 10 Apr. 1989.

The planting date for maize was 5 Apr. 1989 and 26 Apr. 1989 at Fumesua and Pokuase, respectively. For cassava, the planting dates were 10 Apr. 1989 and 27 Apr. 1989 at Fumesua and Pokuase, respectively.

To determine the relationship between the levels of plant density and yield, the component plant population responses were obtained by plotting the yield of the component against its own population expressed over the whole intercropping area. The other crop's component population density was ignored, as suggested by Willey (1979).

Results and discussion

Intercropped-maize
Trial 1

At Fumesua, the following pattern of yield was obtained with 'Dobidi'. While the weight/cob and yield/plant of intercropped maize decreased with increasing density, the grain yield increased with increasing density, reaching a peak at 30,000 plants ha^{-1} (Fig. 1), after which it changed very little. This asymptotic relationship between grain yield and plant density reached after 30,000 plants ha^{-1} suggests that the steep decline in the weight/cob and yield/plant was compensated for by the increasing number of plants as the plant population density was increased from 30,000 to 67,200 plants ha^{-1}, with hardly any influence exerted by No. of ears/plant. A similar pattern was obtained at Pokuase in 1986 (Fig. 2).

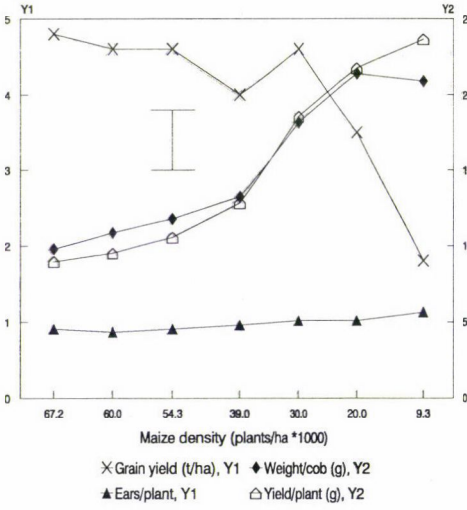

Fig. 1. Yield and components as affected by maize density at Fumesua in 1986. I = twice the SE for maize grain yield

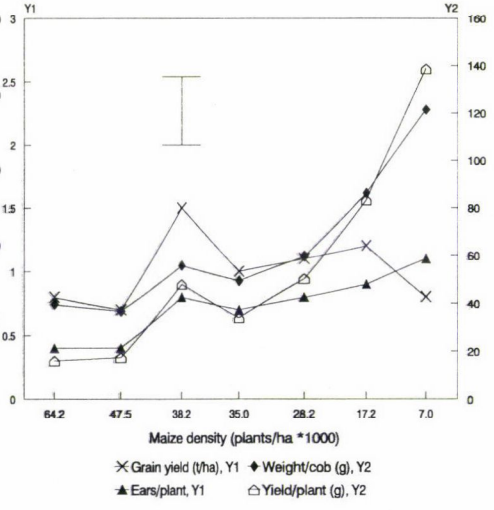

Fig. 2. Yield and components as affected by maize density at Pokuase in 1986. I = twice the SE for maize grain yield

Trial 2

No significant differences were obtained on increasing the plant density as regards the intercropped maize grain yield at Pokuase in 1987, presumably because of the poor weather conditions which prevailed during the major rainy season (Table 3). The rainfall amounts recorded at Fumesua were similar to those at Kwadaso and are not presented. At Kwadaso, however, the grain yield increased, often significantly, as the plant population increased, reaching a peak at 47,700 plants ha^{-1}, so the observed decline in the yield components appeared to have been more than compensated for by the increasing number of plants up to that density (Fig. 3). This increase, however, seems to have been characteristic of the 'Dobidi' + 'Ankra' combination but not of the 'Dorke' + 'Bosome Nsia' combination (Fig. 4), for which the grain yield reached a peak at 38,500 plants ha^{-1} and declined thereafter.

Table 3
Monthly total rainfall (mm) for Kwadaso and Pokuase (1986–1989)

Month	Kwadaso				Pokuase			
	1986	1987	1988	1989	1986	1987	1988	1989
Jan.	0	12	0	52	0	4	0	0
Feb.	100	74	0	0	64	3	9	4
March	102	114	32	170	66	22	80	39
Apr.	178	230	119	95	20	25	51	133
May	214	66	128	124	144	62	242	110
Jun.	198	238	357	397	61	17	255	149
Jul.	162	178	98	50	33	18	98	75
Aug.	26	144	13	143	0	79	12	12
Sept.	60	352	145	227	30	276	31	46
Oct.	196	120	162	201	83	83	130	86
Nov.	24	0	8	62	39	9	54	4
Dec.	20	0	0	3	6	42	28	0

Trial 3

The results obtained at Fumesua in 1989 are presented in Table 4.

While the 1000 grain weight did not influence the grain yield, the other components, especially the number of grains/ear, were often significantly related to yield as shown in Figures 5 and 6. The results suggest that increasing the number of plants beyond 35,000 plants ha^{-1} would not compensate adequately for the sharp decline in the number of grains/ear with increasing density. The grain yield of intercropped maize at Pokuase in 1989, unlike at Fumesua, did not change with density.

Table 4
Yield and yield components of intercropped maize,'Dobidi', as influenced by maize density at Fumesua in 1989

	Grain yield+ (t ha^{-1})	Plant density plants ha^{-1}	Ears/plant	No. of grains/ear	1000 grain wt. (g)
	2.5	19,460	1.00	541.9	236.9
	2.7	34,820	0.89	468.2	213.8
	2.3	42,300	0.88	434.1	224.6.
	2.0	42,070	0.82	440.1	211.8.
	1.8	60,800	0.74	361.2	213.1
CV%	22.8	14.0	10.2	11.4	9.3
SED	0.2	2.632	0.04	24.2	n.s.

+Average yield of maize monocrop at 20,000 and 40,000 plants ha^{-1} = 2.6 tons ha^{-1}, +Significant at P = 0.05, n.s. = non significant

Intercropped cassava

Trial 1

For intercropped cassava, the tuber number did not vary significantly with density but the tuber weight decreased with density at Fumesua (Fig. 7). Work at CIAT (1972; 1973) suggests that a decrease in tuber yield at high plant population densities is attributable to a decrease in tuber weight rather than to tuber number per hectare.

The results shown in Figure 7 demonstrate that the cassava tuber yield did not decrease with increasing plant density, but rather increased with density up to 8,000 plants ha^{-1}, and again from 12,800 to 20,000 plants ha^{-1} at Fumesua. The expected decline in tuber yield with density did not occur, probably because the yields recorded included substantial numbers of small-sized, unmarketable tubers that characterized the storage tuber production at high densities. This proliferation of poorly filled tubers was certainly at the expense of the larger-sized, saleable tubers. Similar results were obtained at Pokuase in 1986 (Fig. 8).

Trial 2

When harvesting was carried out at Pokuase 9 months after planting (MAP), the highest yield was recorded at 10,400 plants ha^{-1} (Fig. 9). Harvest at 12 MAP produced a similar trend in the results (Fig. 9), but a second peak was recorded at 20,400 plants ha^{-1} (Fig. 10) instead of 15,600 plants ha^{-1} as observed at 9 MAP. Cock et al. (1977) have reported that the optimum densities for the varieties change with the age at harvest. The cassava tuber yield at Kwadaso in 1987, however, did not vary with the plant density.

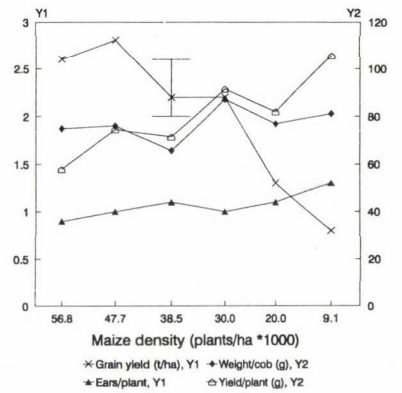

Fig. 3. Yield and components as affected by maize density at Kwadaso in 1987. I = twice the SE for maize grain yield

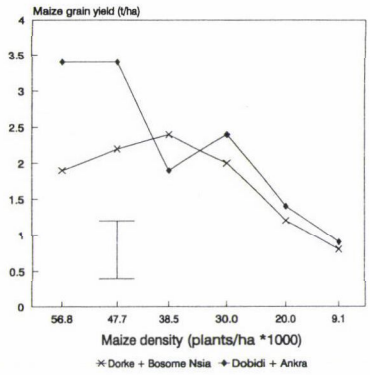

Fig. 4. Yield as affected by maize density and by intercrop at Kwadaso in 1987. I = twice the SE for maize grain yield

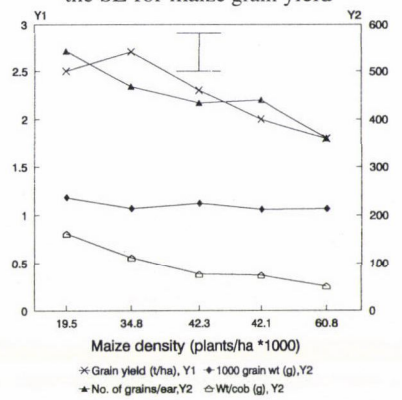

Fig.5. Yield and components as affected by maize density at Fumesua in 1989. I = twice the SE for maize grain yield

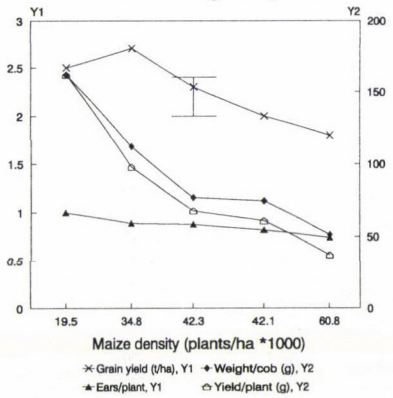

Fig. 6. Yield and components as affected by maize density at Fumesua in 1989. I = twice the SE for maize grain yield

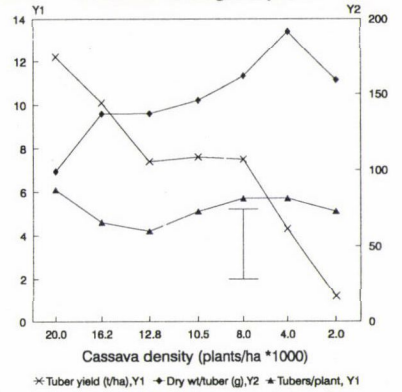

Fig. 7. Yield and components as affected by cassava density at Fumesua in 1986. I = twice the SE for wet cassava yield

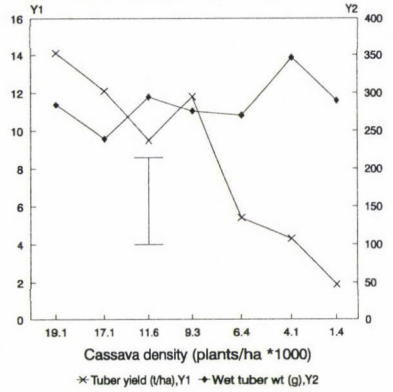

Fig. 8. Wet tuber weight as affected by cassava density at Pokuase in 1986. I = twice the SE for cassava yield

Trial 3

At Fumesua, the cassava tuber yield was maximum at about 42, 070 plants ha^{-1} maize density and decreased significantly with a further increase in maize density (Table 4). The yield components also seemed to decrease with the increasing density of intercropped maize beyond 42,000 plants ha^{-1} (Table 5). There was not much response to the treatments at Pokuase.

Table 5
Yield and yield components of intercropped cassava ('Ankra') as influenced by maize density at Fumesua in 1989

	Dry tuber yield+ (t/ha)	Maize plant density (plant/ha)	Tubers /plant	Weight /tuber (dry) (g)	Crown depth (cm)	Diameter (cm) Crown	Diameter (cm) Tuber	Plant height (cm)
	2.7	19,460	3.5	73.9	163.1	112.6	4.97	251
	2.1	34,820	3.2	62.2	155.7	105.1	4.40	238
	2.3	42,300	3.1	73.7	154.6	118.0	4.56	242
	3.1	42,070	3.6	82.3	147.7	112.9	4.89	237
	2.0	60,800	3.0	71.9	136.6	93.8	4.33	219
CV%	28.3		26.0	23.9	11.7	14.1	8.10	9.9
SED	0.3		n.s.	n.s.	8.4	7.2	0.18	n.s.

+Significant at P=0.05, +Yield of cassava monocrop at 10,000 plants ha^{-1} = 4.3 t ha^{-1}, n.s. = non significant

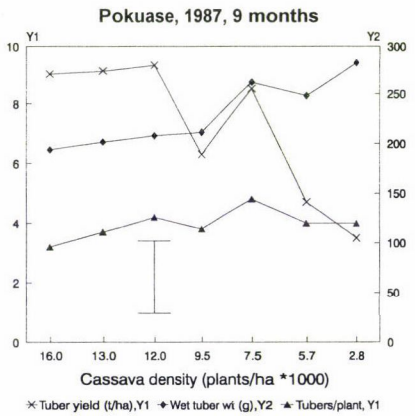

Fig. 9. Wet tuber yield and components as affected by cassava density. I = twice the SE for wet cassava yield

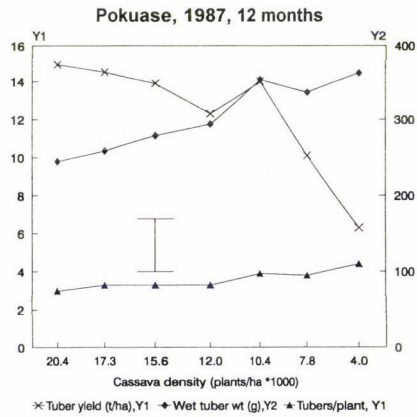

Fig. 10. Wet tuber yield and components as influenced by cassava density. I = twice the SE for wet cassava yield

Conclusions

The results suggest that an intercrop of maize and cassava, with a high level of temporal complimentarity in differences in maturity, respond to component populations as high as their sole crop optimum populations through an efficient use of early season and late season growth resources. The growth patterns of the two crops interfere minimally because of a phase difference in their peak demand for growth factors (IITA, 1982; 1983). The intercropped environment is, therefore, efficiently utilized and the yield of each component is good, which presumably accounts for the widespread adoption of this technique. The yields obtained were highest at the respective near-optimum populations of the component crops. In Ghana, cassava is monocropped at 10,000 plants/ha while maize is monocropped at 62,500 plants/ha.

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Short communication

DETERMINATION OF CRITICAL LIMIT WITH BUILT-UP LEVELS OF MANGANESE FOR OATS (*Avena sativa*) GROWN ON LOAMY SAND

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A pot experiment was conducted on a Mn-deficient loamy sand to determine the critical deficiency limit of Mn for predicting the response of oats to Mn application. There were 15 rates of Mn (0 to 600 mg Mn kg^{-1} soil). The dry matter yield of oat plants increased with successive doses of Mn, the maximum being 23.8 g pot^{-1}, after which it declined non-significantly with an increase in the level of added Mn. Soil Mn was significantly related to plant Mn uptake (r = 0.98**), indicating that DTPA extractant was a good indicator of soil Mn status. The graphical and statistical procedures of Cate and Nelson indicated the critical limit to be 4.5 mg kg^{-1} soil of DTPA-extractable Mn. The critical deficiency limit of Mn in the youngest mature leaf blade at the boot stage of plant growth was 19.0 μg g^{-1} dry matter.

Key words: oats, critical limits, Mn deficiency symptoms, Mn uptake

Introduction

Oats *(Avena sativa)* are grown mainly in the temperate region. In India, they are grown in winter for use as fodder. This crop is a highly nutritious, palatable, non-legume fodder accepted by all kinds of livestock, but is specially suitable for dairy animals and horses. Besides their cultivation for fodder purposes, oats and various oat products are being increasingly used for food, medicine and other industrial purposes. The yield of oats is often low. The poor yield of this crop, despite the adequate application of NPK fertilizer, has been ascribed to the inadequate supply of micronutrients. Manganese deficiency has been recognized as next in importance to that of zinc in the coarse-textured soils of Punjab, India. Therefore, the establishment of the critical limits of Mn in soil and plant is a pre-requisite for separating Mn-deficient from non-deficient soils. In most of the work published so far, manganese deficiency in oats grown on organic soils has been reported (Reid, 1982; Karamanos et al., 1984), but information is lacking regarding the critical limit of available Mn for predicting the profitable response of oats to Mn application in coarse-textured Typic Ustochrepts. Since the diethylene triamine penta acetic acid (DTPA) method of Lindsay and Norvell (1978) has been found to be the most suitable and efficient in predicting the response of oats to applied Mn for neutral and alkaline soils in India (Gangwar and Singh, 1992), this extractant was employed for determining

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the available Mn status of the soils. The present study was, therefore, undertaken to determine the response and critical limits of Mn in coarse-textured sandy soils and in oat plants.

Materials and methods

The pot culture experiment was conducted in a greenhouse in a Mn-deficient soil from Ludhiana, India (30°56' N and 75°32' E, 247 m above mean sea level). The soil was a loamy sand belonging to the group Ustochrepts. It had pH 8.2, electrical conductivity 0.2 dS m^{-1} at 25°C (1:2 soil:water suspension), organic carbon 0.36%, available P 9.0 mg kg^{-1}, K 86 mg kg^{-1} and available manganese extractable by DTPA 2.6 mg kg^{-1} soil. The content of available Fe estimated by DTPA was 4.8 mg kg^{-1} soil. Earthen pots of 5 kg capacity were filled wih 4 kg soil. The treatments consisted of 15 rates of Mn (0, 2.5, 5, 10, 15, 20, 40, 60, 80, 100, 200, 300, 400, 500 and 600 mg kg^{-1} soil) supplied in the form of manganese sulphate solution. All the treatments were replicated four times in a completely randomized design. After incubating the soil at 80% of the moisture content at 1/3 bar tension for a period of 4 weeks, soil samples were taken from each pot to estimate the available Mn in each treated pot before the sowing of the crop. Each pot was given 37 mg N, 7 mg P and 6 mg K kg^{-1} soil as a basal dose in the form of urea and potassium dihydrogen orthophosphate. Ten seeds of oats (Cv. Kent) were sown per pot and were thinned to three plants per pot after germination. The crop was harvested at the boot stage of plant growth (Large, 1954).

The youngest mature leaf blades from each pot were sampled at the time of harvest and the remaining plants were removed by cutting at the soil surface. The plant samples were washed with 0.1 N HCl, distilled and deionized water, dried at 70°C and ground in a stainless steel mill to pass through a 20-mesh sieve. The plant material was wet ashed in a nitric-perchloric-sulphuric acid mixture. The soil samples were analysed for their initial available Mn and Fe content by extraction with a 0.005 M DTPA buffer solution (diethylene triamine penta acetic acid containing 0.1 M triethanol amine and 0.01 M $CaCl_2$ adjusted to pH 7.3 with distilled HCl) using a soil:solution ratio of 1:2 and a shaking time of 2 hours (Lindsay and Norvell, 1978). The manganese content in soil and plant extracts was determined by atomic absorption spectrophotometry. The organic carbon in the soil sample was estimated by the Walkley and Black (1934) rapid titration method. In this method, a 2 g soil sample is treated with 10 ml 1 N $K_2Cr_2O_7$ solution in the presence of 20 ml concentrated H_2SO_4. The soil is slowly digested at low temperature by the heat of dilution of H_2SO_4, and the organic carbon in the soil is oxidized to carbon dioxide. The excess of $K_2Cr_2O_7$, unused in oxidation, is titrated back against a standard solution of 0.5 N ferrous ammonium sulphate in the presence of 3 g sodium flouride, 100 ml distilled water and 10 drops of diphenylamine indicator. NaF makes the change in colour distinct because of its focculating effect. At the end point, the colour of the suspension changes from violet, through blue, to bright green. The phosphorus in the soil sample was determined by extracting the soil with 0.5 N $NaHCO_3$ (pH 8.5) by the method of Olsen et al. (1954) and exchangeable K by using 1 N ammonium acetate solution (pH 7.0). The critical deficiency levels of Mn in soil and plant were estimated by the procedures of Cate and Nelson (1965, 1971).

Results and discussion

Visual symptoms of Mn deficiency were observed in the non-manganese treatment. The leaves exhibited grey specks and lesions, breaking over near the middle, while the older leaves died off.

Dry matter yield and Mn uptake

The dry matter yield of oats increased with an increase in the level of DTPA-extractable Mn, and a significant increase compared with the control was recorded at 4.1 mg Mn kg^{-1} soil (Table 1). The highest increase in dry matter yield was recorded at 4.9 mg Mn kg^{-1} soil, beyond which the yield started declining. The dry matter yield at the highest level of Mn (39.8 mg kg^{-1} soil) decreased non-significantly from the maximum, but it remained higher than in the control. The enhanced supply of Mn resulting from the increasing rates added to the Mn-deficient soil was responsible for the observed increase in yield. This is substantiated by the significant increase in the concentration of Mn in the youngest mature leaf blades as well as in the whole plant. The manganese concentration in the youngest mature leaf blades was found to increase progressively with the increase in DTPA-Mn, increasing from 10.5 μg g^{-1} in the control treatment to 117.0 μg g^{-1} at the highest level of DTPA-Mn. Also, the concentration of Mn in the whole plant was 17.3 μg g^{-1} in the non-manganese treatment, increasing to 170.0 μg g^{-1} with the application of Mn. The percentage increase in dry matter yield with the increase in Mn levels varied from 2.1 to 49.0 (Table 1).

Table 1
Effect of Mn on the yield and Mn uptake of oats

No.	Treatment Mn (mg kg^{-1} soil)	DTPA extractable soil Mn (mg kg^{-1} soil)	Dry matter yield (g pot^{-1})	% of maximum yield	Mn conc.in index leaf (μg g^{-1} dry wt.)	Mn conc.in whole plant (μg g^{-1} dry wt.)	Mn uptake (μg pot^{-1})
1	0.0	2.6	18.9	79	10.5	17.3	326
2	2.5	2.8	19.3	81	12.0	20.3	392
3	5.0	3.2	20.0	84	14.5	21.5	429
4	10.0	3.8	20.8	87	16.0	24.6	512
5	15.0	4.2	22.9	96	18.5	25.6	587
6	20.0	4.9	23.8	100	19.5	29.1	693
7	40.0	7.7	23.7	99	21.5	37.6	750
8	60.0	10.2	22.8	96	23.5	38.3	873
9	80.0	11.3	22.1	93	25.0	48.1	1062
10	100.0	12.5	21.6	91	29.0	72.1	1558
11	200.0	17.6	21.4	88	35.0	74.0	1583
12	300.0	23.8	20.7	87	37.5	82.0	1699
13	400.0	29.6	22.0	92	44.0	89.0	1957
14	500.0	36.1	21.0	88	80.0	137.0	2880
15	600.0	39.8	20.8	87	117.0	170.0	3489
	$LSD_{00.5}$		3.8				716

The Mn uptake by oats increased with the increase in Mn levels in the soil, ranging from 326 to 3489 μg pot^{-1}. There was a 1.2- to 10.7-fold increase in soil Mn. There was a significant coefficient of correlation between Mn uptake and the Mn concentration in the youngest mature leaf blades (r = 0.95**), as shown in Table 2, suggesting that the concentration of Mn in the leaf blade reflects the Mn status of the crop.

Table 2
Linear coefficient of correlation between different plant and soil parameters

Parameters	'r' value	R^2 (%)
DTPA Mn × Mn uptake in plant	0.98*	96.0
DTPA Mn × Mn concentration in index leaf	0.91*	82.8
Mn uptake in plant × Mn concentration in index leaf	0.95*	90.2

* significant at 1% level

Critical levels of Mn in soil and plant

The DTPA-extractable soil Mn was significantly correlated with the Mn concentration in the youngest mature leaf blades (r = 0.91**), as well as with Mn uptake (r = 0.98**), indicating that the DTPA-extractable Mn was a good indicator of soil Mn status. The method described by Cate and Nelson (1965) was used to determine the critical deficiency levels in soil and plant. This method consists of plotting the dry matter yield expressed as a percentage of the maximum yield versus soil Mn. A cross is placed over the data and moved until the upper left and lower right quadrants have a minimum number of points (Fig. 1). The critical value is read from the X-axis where the cross intercepts it. This value was also calculated using the statistical model of Cate and Nelson (1971). Both the approaches gave the same critical value of 4.5 mg Mn kg^{-1} in soil. Using the same technique, the critical deficiency limit in the youngest mature leaf blade at the boot stage of plant growth was found to be 19.0 μg g^{-1} dry matter. A perusal of the data in Table 1 also reveals a much lower yield in treatments where the concentration in the leaf blade was below 19.0 μg g^{-1} than in those where it was above this value. The critical deficiency limit of Mn has been reported to differ markedly for different crops in the same soil. Nayyar et al. (1985) and Bansal et al. (1987) reported 3.5 and 2.05 mg kg^{-1} DTPA-extractable Mn for wheat and barley in Ustipsamments and Ustifluvents soils, whereas in alluvial soils of the Typic Hapludalf type a critical limit of 6.8 mg kg^{-1} DTPA-Mn was found by Gangwar and Singh (1992) for predicting the response of oats to Mn.

Critical deficiency levels of Mn in oat tissue have been set by a number of investigators. Hammes and Berger (1960) reported a critical deficiency level of 15 μg g^{-1} using the same plant sampling technique. A critical deficiency level of

28.0 μg g^{-1} in the whole plant at 60 days was reported by Gangwar and Singh (1992). A critical toxicity level of 500 μg Mn g^{-1} in recently matured leaves has been reported (Jones, 1971). However, in the present study the concentration of Mn at the boot stage ranged from 17.3 to 170.0 μg g^{-1} in whole oat plants and from 10.5 to 117.0 μg g^{-1} in leaf blades, which is well below the toxic level reported above. Thus, the present investigations suggest that oat plants having a Mn concentration below the critical limit of 19.0 μg g^{-1} in the youngest mature leaf blade in the boot stage and loamy sand soil with a DTPA-extractable Mn level below 4.5 mg kg^{-1} may be rated as deficient and will need Mn fertilization to yield at the desired level.

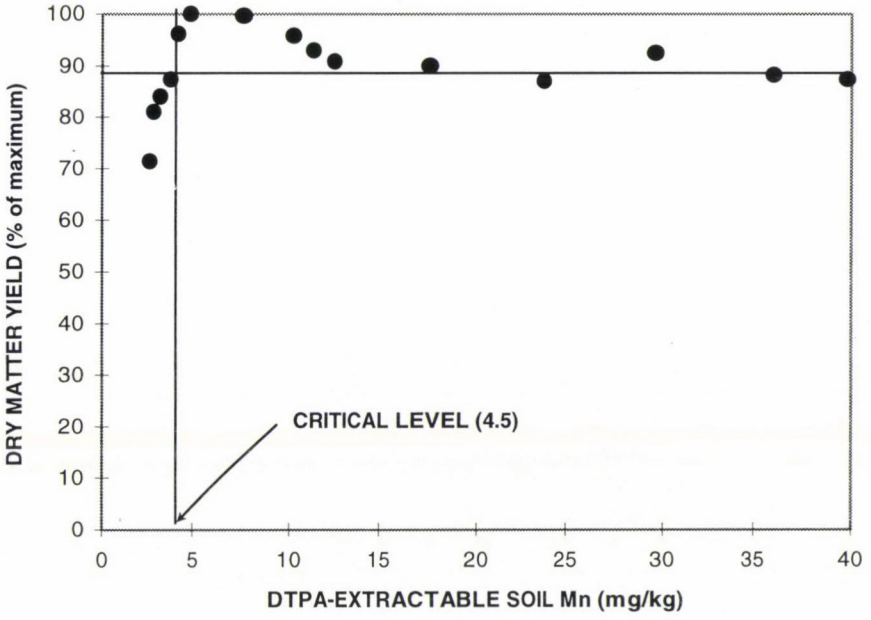

Fig. 1. Scatter diagram of dry matter yield (% of the maximum) versus DTPA extractable soil Mn

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Review

USE OF CHLOROPHYLL FLUORESCENCE INDUCTION TECHNIQUES IN THE STUDY OF LOW TEMPERATURE STRESS IN PLANTS

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Low temperature may cause a severe decrease in the photosynthesising ability, especially in chilling-sensitive plants. The chlorophyll fluorescence induction technique provides a non-destructive method for investigating the photosynthetic apparatus of plants. Partly due to a technical improvement over the last decade, this technique has become a widely used method in basic and applied research. This study discusses the effects of one of the most important crop limiting factors, low temperature stress, on a number of chlorophyll fluorescence induction parameters in the fast and slow phases of the fluorescence induction curve, on quenching components and on the F685/F730 ratio. The investigations covered both the temperature dependence of the chlorophyll fluorescence induction parameters and the effect of low temperature stress and low temperature-induced photoinhibition on these parameters.

Key words: chilling, chlorophyll fluorescence induction, frost tolerance, photosynthesis

Introduction

Low temperature stress is one of the most important factors limiting the wider spread of several crop plants. Numerous stress factors, such as herbicides, have a primary site of action which can be fairly well defined, whereas low temperature affects practically all the processes involved in the metabolism. In the case of plants originating from tropical or subtropical regions (e.g. maize, tomato, etc.) even temperatures of below 10–15°C may cause considerable damage. Especially in the case of C_4 plants photosynthetic processes are disturbed by low temperature. By contrast, in many species low, non-freezing temperatures have an important physiological function. In the case of winter wheat, temperatures in the 0–5°C range are essential partly for vernalisation and partly for the development of frost resistance and winter hardiness. For these species serious damage is only likely to occur after long periods of freezing temperatures.

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Chlorophyll fluorescence induction provides rapid, quantitative, non-invasive methods for following changes in the photosynthetic apparatus. This technique is used not only in basic photosynthesis research but also for investigating the effects of various biotic and abiotic stress factors.

The present paper aims to provide a review of changes occurring in the most frequently applied chlorophyll fluorescence induction parameters in the course of low temperature stress.

Chlorophyll fluorescence induction

The pigments involved in photosynthesis reach a state of excitation as the result of absorbed light energy. They can return to their ground state either by photochemical reactions in the reaction centres, through energy transfer to other pigments in the antenna system or due to deactivation involving heat or light emission.

If photosynthesising plant material is kept in the dark for a sufficient length of time and then illuminated, the fluorescence intensity can be seen to reach the equilibrium level through well-defined transient levels (Fig. 1). This phenomenon is known as the Kautsky effect after the scientist who first described it (Kautsky and Hirsch, 1931). When dark-adapted samples are illuminated the fluorescence jumps within a few ps to an initial level (F_o), then reaches a maximum (F_p) after passing through transient levels I and D. At saturation light intensity F_p is equal to the maximum fluorescence (F_m). The difference between F_m and F_o gives the variable fluorescence (F_v), which is proportional to the efficiency of the light energy utilization of PSII. Since the fluorescence reaches the F_p level within 1–2 s, the interval from F_o to F_p is known as the fast phase of fluorescence induction. If illumination is constant, the fluorescence level begins to drop due to various quenching mechanisms and, after passing through one or more local maximum (M) and minimum (S) levels, generally reaches a steady state (T) after approx. 10–15 min. The interval from P to the steady state is known as the slow phase of the induction curve.

In recent years the spread of the PAM fluorometer has made it possible to determine photochemical (q_P) and non-photochemical (q_N) quenching (Schreiber et al., 1986). The essence of this method is that during illumination with photochemically utilisable (actinic) light the sample is exposed to a flash of saturation intensity light which, by saturating photosynthesis, excludes the possibility of photochemical quenching. The fluorescence increment obtained after the saturating flash (ΔF) will be proportional to the photochemical quenching, while the difference between this and maximum fluorescence will be proportional to the extent of non-photochemical quenching. Non-photochemical quenching includes three further components: the main component, the mechanism involved in energy-dependent quenching (q_E), has a short relaxation half-life (<1 min), the relaxation time of the "middle" component (q_T), involved

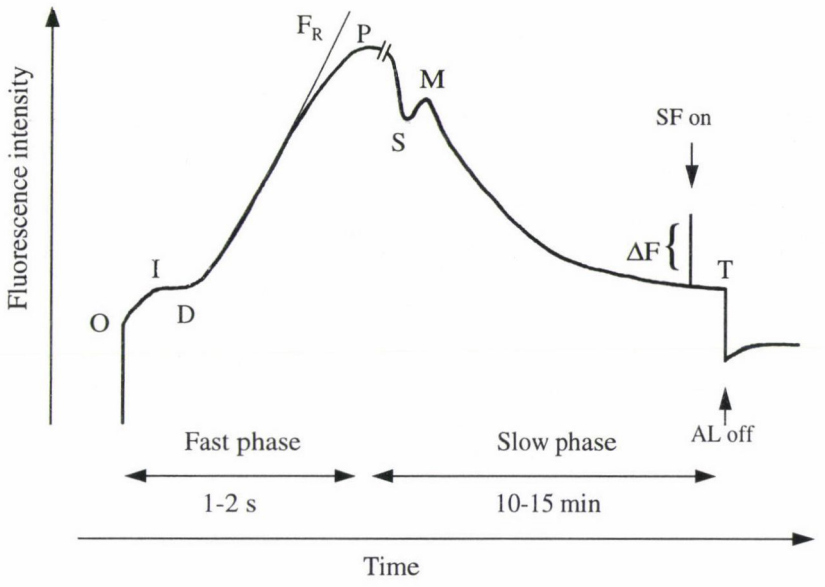

Fig. 1. Schematic chlorophyll fluorescence induction curve in a dark adapted leaf under illumination. F_R: maximal rate of the induced rise of fluorescence; SF: saturation flash; AL: actinic light. (For details see text.)

in the transition from state I to state II, is approx. 6–8 min, while photoinhibition (q_I) is responsible for the "slow" component ($t_{1/2}$>30 min) (Bilger and Schreiber, 1986; Hodges et al., 1989; Walters and Horton, 1991). [For further details on the phenomenon of chlorophyll fluorescence and on fields of application, see Szigeti (1989), Krause and Weis (1991), Lichtenthaler (1992) and Bolhár-Nordenkampf and Öquist (1993).] For the chlorophyll fluorescence induction parameters the nomenclature of van Kooten and Snel (1990) was used.

Due to the development of instrumentation using a charge-coupled device and an optical multichannel analyser (CCD OMA) the spectral composition of the emitted fluorescence during the fast rise and slow decay can be followed and analysed (Szigeti et al., 1996).

Chlorophyll fluorescence and chilling stress

Fast phase

Low, but non-freezing temperature itself (i.e. when the induction curve is recorded at low temperature in unchilled control plants) has no significant effect on either F_o or F_v as demonstrated for example for spinach (Klosson and Krause, 1981), pea (Georgieva and Yordanov, 1993) and wheat plants (Janda et al., 1994a). By contrast, there is a decrease in the F_v (Georgieva and Yordanov, 1993) and an increase in the F_o at high temperatures (Ducruet and Lemoine,

1985). In the case of the chilling-sensitive species tomato temperature again had no significant effect on F_o and F_i between 5 and 30°C, and there was less than 10% reduction in F_m (determined using saturating pulse) at 5°C compared to the value measured at 21°C. When F_m was determined at low actinic light using DCMU [3-(3,4-dichlorophenyl)-1,1-dimethylurea, an inhibitor of electron transport between Q_A and Q_B], F_m showed a slight increase from 22°C to 0°C (Janssen and van Hasselt, 1994). F_p measured at low actinic light intensity showed 3 phases between 30°C and 5°C (Janssen et al., 1992). It increased from 30°C to 22°C, decreased from 22°C to 14°C and then increased again. Q_A reduction calculated with the $(F_p - F_o)/(F_m - F_o)$ equation (Dietz et al., 1985; Schreiber et al., 1986) showed a similar pattern (Janssen and van Hasselt, 1994).

Temperature can affect the excitation energy distribution by changing the distribution of the $PSII_\alpha$ and $PSII_\beta$ centres (Sundby et al., 1986; Govindjee, 1990). $PSII_\alpha$ reaction centres are found in the grana regions of the chloroplast thylakoid membranes, and have a bigger light harvesting system than $PSII_\beta$ centres, which are located in the stroma region (Melis, 1985). In leaf discs treated with DCMU it is possible to investigate the heterogeneity of PSII (Melis and Homann, 1975). The proportion of $PSII_\alpha$ to $PSII_\beta$ in tomato plants did not change significantly between 22°C and 3°C, but decreased when the temperature was raised to 30°C (Janssen and van Hasselt, 1994).

The F_v/F_m parameter is used most frequently to express the maximum photochemical efficiency of PSII. (The parameters F_v/F_o and F_m/F_o have much the same meaning and provide a better illustration of the relationship of variable and maximum fluorescence to F_o.) After low temperature stress in the light a strong decrease occurs in F_v/F_m (Hetherington et al., 1989; Schapendonk et al., 1989; Janda et al., 1994b,c). Although this decrease is mainly due to a decrease in F_v, an increase in the initial fluorescence (F_o) can also be observed (Schapendonk et al., 1989; Janda et al., 1994b). The decrease in F_v/F_m is affected not only by the temperature, but by the irradiance during the cold treatment as well: the higher the irradiance the more pronounced the decrease in this parameter (Janda et al., 1994b,c). When cold treatment was carried out in complete darkness there was no change in the F_v/F_m parameter even after 2 days at 5°C, while in the light this parameter continuously decreased. When a maize plant cold-treated for 24 h in the light was exposed to cold for a further 24 h in the dark, the decrease in the F_v/F_m ratio was halted. At 25°C in the light this parameter recovered, and regained its initial value (Janda et al., 1994b). These results can be explained by the theory that low temperature hinders the migration of newly synthesised D1 protein within the thylakoid membrane (Kyle, 1987).

After dark cold treatment a significant change in F_v/F_m can only be detected after extremely severe stress conditions. In young maize plants this parameter only showed a significant decrease after 5 days at 0.5°C in the dark

(Szalai et al., 1996). However, F_v/F_m showed a sudden decrease when the plants were returned to non-chilling temperatures, and the level of this decrease depended on the irradiance: it was more pronounced in the light than in the dark. These results suggest that photoinhibition (i.e. damage to the photosynthetic apparatus caused by higher light intensity than that which can be utilised in photosynthesis or can be dissipated in harmless processes) has a role not only during low temperature stress, but in post-chilling symptoms as well (Janda et al., 1996; Szalai et al., 1996).

The initial or minimum fluorescence (F_o) often increases after several types of stresses which cause damage to Photosystem II (Demmig and Björkman, 1987). A slight increase in F_o was observed after the cold treatment of maize plants (Janda et al., 1994). Since the extent of F_o depends on the chlorophyll content (Lichtenthaler, 1988), F_o/chlorophyll content is also used as an indicator of the stress effect. After long-term cold stress a good correlation was found between the increase in this parameter and the cold tolerance of some inbred maize lines (Csapó et al., 1991). An increase in initial fluorescence (F_o) in the course of light stress has been observed in many cases, but not all, during light treatment at both 20°C and at low temperature (Demmig and Björkman, 1987; Somersalo and Krause, 1988; 1989; 1990; Osmond et al., 1993; Krause, 1994a). Some authors are of the opinion that the increase in F_o is a sign of damage to PSII due either to the inactivation of the reaction centre or to the loss of the antenna, while a drop in F_o could be the result of a regulatory or defence mechanism (Demmig and Björkman, 1987; Osmond et al., 1993). These suggestions cannot be generalised, however; in the case of spinach leaves, for example, light treatment at 4°C led to a considerable increase in F_o, but this change exhibited rapid reversibility at both 4°C and 18°C, so it is unlikely to be the result of damage (Somersalo and Krause, 1988). The development of stable Q_A^- may also lead to a reversible increase in F_o, as can be observed under anaerobic conditions with intense illumination (Krause, 1994a).

The F_v/F_m parameter is often used as a screening method for chilling tolerance in several plant species, for example maize (Bergantino et al., 1995; Csapó et al., 1991; Tollenaar et al., 1991; Verheul et al., 1995), rice (Sthapit et al., 1995), potato (Kristjansdottir and Merker, 1993), soybeans (Neuer and Larcher, 1991) and grapevine (During et al., 1990). However, other authors found no close correlation between changes in F_v/F_m and chilling tolerance (Janda et al., 1994c; Haldimann, 1997).

Some authors used the F_R parameter to determine the level of chilling damage. F_R represents the maximal rate of the induced rise in chlorophyll fluorescence during the fast induction phase. This parameter declined as the leaves of maize plants kept at 0°C became injured at low temperature (Hetherington et al., 1983). The decrease in F_R during chilling was much smaller in populations which were hardened before chilling than in unhardened

populations (Hetherington et al., 1983; Verheul et al., 1993). Other authors also found a correlation between this parameter and the chilling tolerance of inbred maize lines grown at suboptimum temperatures (Csapó et al., 1991; Kovács et al., 1992). However, similarly to the F_v/F_m parameter, F_R decreased only after very severe chilling stress in the dark; furthermore, the decrease in F_R reported due to low temperature stress showed a good correlation with the decrease in F_v/F_m (Csapó et al., 1991). The temperature dependence of F_p and the maximum slope of fluorescence induction determined between F_i and F_p were similar, except below 6°C, where there was no correlation between F_p and F_R, probably due to an impaired electron donation to PSII (Janssen and van Hasselt, 1994).

Slow phase

The pulse amplitude modulation technique provides an easy way of determining the photochemical and non-photochemical fluorescence quenching components (Schreiber et al., 1986) and the quantum yield of Photosystem II (Genty et al., 1989). An increase in non-photochemical quenching (or E-quenching, according to an earlier nomenclature indicating q_E as a main component of q_N) was observed in maize plants after low temperature stress (Schapendonk et al., 1989). This increase may be due to a restraint in the CO_2 assimilation, causing a surplus of ATP and a high proton gradient across the thylakoid membranes of the chloroplasts. The decrease in the CO_2 assimilation can be explained by a decrease in the stomatal conductivity (Szalai et al., 1997), a decrease in the activity of the enzymes responsible for the Calvin cycle (Brüggemann et al., 1994) and the C_4 reaction pathway (Stamp, 1987) or the increased photoinhibition of Photosystem II at low temperatures (Long, 1983). There was no significant change in non-photochemical quenching after 1 day of cold treatment at 13°C in a chilling-tolerant genotype, which is assumed to be able to sustain an intense carbon metabolism at low temperatures (Schapendonk et al., 1989).

Photochemical quenching (q_P, or Q-quenching) is also affected by low temperature stress. After 3 days of cold treatment a significant increase in q_P was observed in cold-sensitive maize lines (Schapendonk et al., 1989). However, it was also shown that this increase is only temporary: prolonged severe low temperature stress may cause a decrease not only in the variable fluorescence, but also in the q_P. Furthermore, similarly to other chlorophyll fluorescence induction parameters, changes in q_P due to cold treatment are affected not only by temperature, but also by irradiance (Janda et al., 1994b).

When peas (chilling-tolerant) were compared with *Vigna* spp. (chilling-sensitive), q_P showed a temperature maximum at around 30°C, similarly to gross photosynthesis. This maximum could be observed at a lower temperature in peas than in *Vigna* spp. The temperature at which a decrease in q_P occurred was approx. 10°C higher in *Vigna* spp. than in peas (Brüggemann, 1992). Similar

observations were made on tomato species (Brüggemann and Linger, 1994) and maize lines (Havaux, 1987) with different chilling tolerance. A sudden decrease in the q_P was observed in moderate light in both maize (Labate et al., 1990) and *Vigna unguiculata* (Larcher et al., 1990), probably due to a phase transition in the thylakoid membrane causing an impaired electron flow from Photosystem II to Photosystem I, or due to an impairment of plastoquinol oxidation. These results suggest that the temperature dependence of q_P could be a suitable indicator of the ability of the photosynthetic apparatus to tolerate suboptimum temperatures.

Non-photochemical quenching showed a minimum at around 30–35°C (Brüggemann, 1992). This minimum could be observed at lower temperatures in peas than in *Vigna* spp. As the temperature decreased from 30°C there was an increase in q_N. In the cold-sensitive species, however, q_N decreased suddenly below a temperature of approx. 7°C. The components of non-photochemical quenching can be calculated from the dark relaxation of q_N. q_I did not change between 20°C and 40°C but decreased at low temperature. This decrease occurred at a higher temperature in chilling-sensitive plants than in chilling-tolerant ones (Brüggemann, 1992). Comparing tomato species with different cold sensitivity it was observed that the different capacities of the plants to acclimate to suboptimum temperatures were also reflected in the differential sensitivity of the chilled plants towards photoinhibition induced by actinic light (Brüggemann and Dauborn, 1993; Brüggemann and Linger, 1994). However, other authors found that chilling temperatures increased the likelihood of photoinhibition in leaves of both chilling-sensitive and -resistant plants, and although photoinhibition during chilling generally occurred more rapidly in chilling-sensitive plants, this was not related directly to chilling sensitivity (Hetherington et al., 1989, Janda et al., 1994c; Neuer and Larcher, 1991). The fast relaxing component of q_N (including q_T and q_E) showed a sudden drop below a threshold temperature of 13°C for *Vigna* spp. and 7°C for peas, possibly due to an interruption in the electron transport chain caused by photoinhibition and/or due to the chilling sensitivity of violaxanthin de-epoxidase (Bilger and Björkman, 1991), since zeaxanthin, the product of the reaction catalysed by this enzyme, is thought to be the quencher mainly responsible for q_E (Demmig-Adams and Adams, 1990). It was also observed that high xanthophyll cycle activity and pool sizes in response to a severe high light stress in cold-acclimated spinach leaves may protect these leaves against inactivation of the D1 protein (Thiele et al., 1996). The results suggest that chilling-tolerant species are able to utilize the reduction capacity provided by low actinic light even at low temperatures. In the case of chilling-sensitive plants, a decreased capacity of the dark reactions at low temperatures may lead to decreased utilization of ΔpH and an accumulation of redox equivalents causing an increase in the reduction state of Q_A (Brüggemann, 1992; Havaux, 1987).

A good correlation was found between the quantum efficiency of photosynthetic electron transport and carbon assimilation (Genty et al., 1989; Andrews et al., 1995; Edwards and Baker, 1993). The quantum yield is lower at low temperatures, and similarly to F_v/F_m, it decreases after low temperature stress in the light (Janda et al., 1994b). This is not surprising as the quantum yield of the non-cyclic electron transport is the product of q_P and F_v'/F_m' (F_v and F_m measured in the light-adapted state). It was also shown that F_v'/F_m' not q_P is significantly correlated with the quantum yield of the electron transport (Andrews et al., 1995).

During the measurement of the slow chlorophyll fluorescence induction curve in cold-treated maize plants, quenching occurs not only in F_v, but also in F_o (Janda et al., 1994b, c). After 1 day of cold treatment the higher the irradiance and the lower the temperature the more pronounced was the F_o quenching. However, after a longer chilling period in the light, or below a threshold F_v/F_m the F_o quenching decreased, probably due to the extremely impaired electron transport (Janda et al., 1995). It is generally accepted that one of the non-photochemical quenching components may have a role in F_o quenching. Since F_o can be quenched in the light and restored in the dark relatively rapidly (although low temperature may inhibit the increase in quenched F_o in the dark), q_I may play no role in this process. However, both q_T (Bilger and Schreiber, 1986; Hodges et al., 1989) and q_E (Quick and Stitt, 1989; Ruban and Horton, 1994) may be responsible for q_o. Another possible contribution to the quenching of F_o may originate from the presence of inactive centres which are not able to reduce the second quinone acceptor (Q_B non-reducing centres). These centres can be reduced in relatively low light, so the measurement of initial fluorescence could include a contribution from these centres (Horton and Ruban, 1993). Although due to low temperature stress in the light an increase can be observed in the relative level of F_i, which is in close correlation with the number of Q_B non-reducing centres (Melis, 1985), it can be assumed that the F_o quenching in chilled plants is due mainly to other process(es), because it can be detected even at very low measuring light intensity.

F685/F730

Only a few data are available on the relationship between the F685/F730 ratio and the chilling tolerance of plants. (F685 and F730 represent the fluorescence intensity measured at 685 and 730 nm, respectively.) When cold treatment was carried out at moderate light intensities, this parameter decreased rapidly during the first few hours in cold-sensitive *Phaseolus vulgaris* L. plants and then more slowly, while F_v/F_m decreased almost linearly. As the temperature decreased there was a decrease in the F685/F730 parameter in bean plants, but this parameter increased slightly in chilling-resistant pea plants (Agati et al.,

1996). It is difficult to interpret the changes in this parameter. Due to a decrease in chlorophyll content an increase can be observed in F685/730 under several types of stress conditions (Rinderle and Lichtenthaler, 1988), which can be explained by the decreased fluorescence reabsorption at 685 nm (Lichtenthaler et al., 1990). However, short chilling stress does not cause any change in the chlorophyll content (Lipucci di Paola et al., 1992). The decrease in F685/F730 may be the result of the inhibition of Photosystem II after low temperature stress in the light (Agati et al., 1996).

Chlorophyll fluorescence and frost hardening

Even in the case of winter wheat varieties with excellent frost resistance it is important for them to be hardened at temperatures of 0–5°C for a certain length of time before exposure to temperatures well below freezing point (Veisz and Sutka, 1989). Certain chromosomes (5A, 7A, 4B, 5B, 4D and 5D) play an important role in the development of frost resistance (Sutka, 1981; Sutka and Veisz, 1988). However, while genes responsible for the development of frost resistance are being located more and more successfully, very little is still known about the exact mechanism of hardening. The examinations carried out up to now indicate that several physiological and biochemical changes, for example changes in ABA level (Galiba et al., 1993), in nucleic acid synthesis and rRNA processing (Páldi and Dévay, 1977; Páldi et al., 1996; Páldi et al., 1997; Páldi and Szalai, 1997), in polyamine level (Páldi et al., 1993; Rácz et al., 1996), etc., observed during the hardening period, may be involved in the development of frost tolerance. Studies on the frost hardening effect are complicated by the fact that many processes take place at low temperature. Even cold-tolerant plants must adjust from a relatively high temperature to the low, cold-hardening temperature. Furthermore, processes must be initiated which make the plant capable of surviving frost. In the case of winter wheat a third process, vernalisation, is also initiated, which is necessary if the plants are to flower. In many cases it is impossible to distinguish between these processes when the effects of low temperature are investigated (Abromeit et al., 1992).

It is a well-known fact that, especially in the case of cold-sensitive plants, photoinhibition may take place at low temperature even if the illumination is not very strong. According to certain authors the frost hardiness of plants is determined chiefly by their sensitivity to the photoinhibition induced by low temperature (Huner et al., 1989). This is confirmed to some extent by the fact that in the course of low-temperature

hardening a number of cold-tolerant plants (e.g. spinach) have been demonstrated to become more tolerant to photoinhibition (Krause, 1994b; Somersalo and Krause, 1988). Studies on wheat varieties with various degrees of frost resistance (the winter wheat Cheyenne and the spring wheat Chinese Spring) and on their chromosome substitution lines (Chinese Spring/Cheyenne5A, Chinese Spring/Cheyenne7A), however, indicated that the correlation between the degree of photoinhibition arising during hardening, characterised by the decrease in F_v/F_m, and frost resistance is not close (Janda et al., 1994a). Wheat plants may exhibit a down-regulation of photosynthesis during periods of low temperature and high light, as the result of an increase in the dissipation of excitation energy in the photosynthetic apparatus by radiationless decay processes (Groom and Baker, 1992). Therefore, a decrease in F_v/F_m is not attended by damage to the D1 protein of PS II.

It has also been revealed that frost resistance can be developed and studied not only *in vivo* in whole plants, but also in *in vitro* systems, for instance in calli. The results achieved during testing in such systems exhibit a good correlation with the results of *in vivo* frost tests (Galiba and Sutka, 1988). In these systems, however, there can be no question of photoinhibition, so it seems unlikely that sensitivity to photoinhibition plays a decisive role in the development of frost resistance.

Winter rye and wheat cultivars grown at low hardening temperatures showed a higher proportion of oxidized primary quinone acceptor (Q_A), characterized by the q_P value, than plants grown under non-hardening conditions (Öquist and Huner, 1993). A positive correlation was found between the proportion of oxidized Q_A, the increase in the capacity of photosynthesis and the frost tolerance of spring and winter wheat and winter rye plants. These results suggest that the adaptation of the photosynthetic apparatus to low growth temperature is an important factor in the induction of frost tolerance (Öquist et al., 1993). However, an increase in the proportion of oxidized Q_A could not be observed in Chinese Spring/Cheyenne5A or Chinese Spring/Cheyenne7A chromosome substitution lines, in spite of the fact that these chromosomes improved the frost tolerance of the spring wheat (Janda et al., 1994a). The effects of genes localized on these chromosomes on the freezing tolerance of wheat plants are still unknown.

Conclusions

Certain chlorophyll fluorescence induction parameters, for example F_i, q_P or q_N, depend on the temperature. These parameters showed different temperature dependence in plants with different sensitivity to low temperature. Chilling stress has a great effect on the photosynthetic apparatus. However, this

damaging effect depends not only on the temperature, but on the irradiance as well. Several chlorophyll fluorescence induction parameters (for example F_o, F_m, q_P and q_o) were shown to be changed only after light cold treatment, and these parameters did not change significantly after cold treatment in the dark. However, since low temperature-induced photoinhibition is one of the most important parts of the chilling injury, chlorophyll fluorescence induction may provide a rapid, non-destructive method for detecting the effect of low temperature stress in several plant species.

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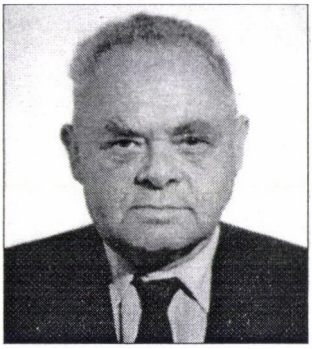
Acta Agronomica Hungarica, 46(1), pp.93–95 (1998)

Obituary

IN MEMORIAM ISTVÁN SZABOLCS
(1924–1997)



The international community of soil scientists has suffered a great loss. Prof. István Szabolcs, the distinguished soil scientist, known and respected internationally, died suddenly - together with his wife Dr. Katalin Darab - on 10 August, 1997.

Prof. Szabolcs was well-known all over the world among pedologists, agrochemists, agroecologists and scientists in related fields for his broad and continuously updated knowledge, brilliant memory and logical thinking, creativity, and unbelievable enthusiasm for his subject and for research. During his career he brought an almost missionary zeal to his work in soil science and especially in the field of salt-affected soils.

István Szabolcs was born in Túrkeve, Hungary, in 1924. He graduated from Debrecen University of Sciences with a B.Sc. in Chemistry in 1948. He obtained his C.Sc. (Ph.D.) degree in Moscow, USSR and his D.Sc. degree in Budapest in 1968, based upon theses entitled "Salt-affected soils in the Hortobágy region (Hungary)" and "The influence of water regularities and irrigation on soil processes in the Transtisza region".

Following two years' service as deputy director of the Irrigation Research Institute in Szarvas (1953–1954) he was appointed first deputy director (1954–1959) and then director (1959–1981) of the Research Institute for Soil Science and Agricultural Chemistry of the Hungarian Academy of Sciences, Budapest. After his retirement he continued to act as scientific adviser to the Institute until his death. He was appointed professor of soil science at Eötvös University, Budapest, in 1968.

His main field of interest was the development, regime, classification, mapping, utilization and amelioration of salt-affected soils. He defined,

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described, characterized and quantified the primary and secondary salt accumulation processes, the mechanisms of phase interactions under saline and/or alkali conditions, the formation of "solonetzes" and "solods" and the changes induced by human intervention in salinization/alkalization/sodification processes. He introduced such important terms as: secondary salinization; critical depth of groundwater table; potentially salt-affected soils. His research team elaborated a comprehensive soil survey – analysis – mapping – monitoring system for the prediction and prevention of salinization/alkalization processes which was efficiently used not only on the Hungarian Plain and in the Carpathian Basin, but in many other countries with similar natural conditions as well. He developed a new classification system for salt-affected soils and prepared maps of these soils for Hungary (1:500,000) and for Europe (1:5,000,000). He coordinated the project "World Map of Salt-Affected Soils".

In the early sixties he was the main promoter of large-scale genetic soil mapping in Hungary and edited the Hungarian handbook for this programme. He took an active part in the Desertification Assessment UNEP Programme, collaborated with IAEA (International Atomic Energy Agency) for five years in isotope tracer applications in soil science and agrochemistry, and in recent years in the various "post-Rio" international projects on soil resilience and sustainable land use.

Prof. Szabolcs published 17 books and more than 630 papers in scientific journals in Hungary and abroad. His monographs on salt-affected soils "European Solonetz Soils and their Reclamation", Akadémiai Kiadó, Budapest, 1971; "Salt Affected Soils in Europe", Martinus Hijhoff, The Hague – RISSAC, Budapest, 1974; "Modelling of soil Salinization and Alkalization", Agrokémia és Talajtan, Tom. 28. Suppl., 1979; "Review of Research on Salt Affected Soils", UNESCO, Paris, 1979; "Salt Affected Soils" CRC Press, Inc., Boca Raton, USA, 1989) are well known all over the world.

In 1964 he organized the International Symposium on Sodic Soils (ISSS) in Hungary. This meeting, with the participation of top experts on salinity/alkalinity from all continents, managed to break through the "iron curtain" and, for the first time in many years, gave the opportunity for direct dialogues and discussions between the various scientific schools on salt-affected soils. The success of the Symposium was one of the reasons for re-establishing the Alkali Subcommission of the ISSS (which existed from the early 30's, under the chairmanship of Prof. Alexius J. 'Sigmond and Prof. S. Arany, Hungary, up to the 2nd World War) during the 8th Congress of ISSS (Bucharest, 1964). He was appointed Chariman of the reactivated Subcommission on Salt-Affected Soils of the ISSS and filled this position until 1982. During (and after) this time he initiated and organized numerous symposiums and scientific meetings (Budapest, Yerevan, Cairo, Novi Sad, Osijek, etc.) and the Subcommission was one of the most active groups of ISSS.

In 1974 (10th ISSS Congress, Moscow) he was elected Deputy Secretary General of the ISSS. He was re-elected to this position at the 11th (Edmonton, Canada, 1978), 12th (New Delhi, India, 1982) and 13th (Hamburg, Germany, 1986) ISSS Congresses. At the 14th Congress (Kyoto, Japan, 1990) he was elected Honorary Member of the ISSS. Dr. Szabolcs participated in all ten ISSS Congresses from 1956 and became a decisive personality in ISSS. He played important roles in other international organizations (UNESCO, UNEP, CICRA, CIEC, etc.) as well. He was the Director of the International Post-graduate Course on Salinity and Alkalinity (Budapest, 1973), promoter of the Indo-Hungarian Seminars on the Management of Salt Affected Soils (Karnal, India, 1977; Budapest, Hungary, 1981), FAO consultant at the Chambal Project (Rajasthan, India, 1969), IIASA consultant in Laxenburg (1982), UNEP consultant at the 1985 Planning Commission Meeting, and invited lecturer in many countries (Ghana and Nigeria, 1969; Tunisia, Kenya, Tanzania, Ethiopia and Sudan 1974; Iraq, 1979; Tunisia, 1980, 1982).

Prof. Szabolcs was President of the Hungarian Soil Science Society between 1970 and 1990, and after two decades of efficient service he was elected Honorary President. He was honorary member of the Indian, Soviet, Russian, Bulgarian and Rumanian soil science societies.

From 1960 he was editor-in-chief of the journal "Agrokémia és Talajtan" (RISSAC, Budapest) and was a member of the editorial boards of Acta Agronomica and of numerous other Hungarian and international scientific journals (Agrochimica, Geoderma, International Journal of Tropical Agriculture, Soil Survey and Land Evaluation).

For his achievements Prof. Szabolcs received two Governmental Awards, the "Tessedik Gold Medal" (Hungarian Agricultural Society) and the "Treitz Medal" (Hungarian Soil Science Society). In 1996 he was awarded the "Dokuchaev Gold Medal", the highest award of the Russian Soil Science Society.

The life of a highly intelligent and eminent scientist came to an end on 10 August, 1997. The scientific achievements of Professor István Szabolcs will remain and will be continued by his colleagues and students. He will be sadly missed by the world's soil scientist community.

G. VÁRALLYAY

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Kiss, G., Papp, I., Bakondi-Zámori, E., Gartner-Bánfalvi, Á. (1977): A szója fungicides magcsávázásának és rhizóbium oltásának együttes tanulmányozása (Joint study of fungicide dressing and rhizobium inoculation in soybean). *Növénytermelés,* **26,** 147–153.

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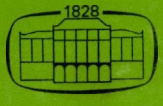

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ORIGINAL PAPERS

REVIEW

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DEVELOPMENT OF PHOSPHINOTHRICIN RESISTANT FERTILE TRANSGENIC TOBACCO PLANTS

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(Received: 20 February, 1998; accepted: 16 April, 1998)

This study was performed to develop transgenic tobacco plants (*Nicotiana tabacum*, Samsun) resistant to broad spectrum herbicides based on phosphinothricin (PPT). The *bar* gene that is responsible for the synthesis of the phosphinothricin N-acetyl-transferase (BAR) enzyme, which detoxifies PPT through acetylation, was transferred to tobacco plants. The LBA4404 strain of *Agrobacterium tumefaciens* harbouring the binary vector pDHB321.1 was used in the transformation experiments. Gene transfer was performed using the leaf disc transformation system. At the end of the transformation experiments, callus stem and root formation were observed at 10 mg/L PPT, which was the lethal dose. Following transfer to soil, the regenerated primary transgenic plants were not affected by the application of 1% (v/v) BASTA® (30 L/ha) preparations equivalent to 2.0 g/L gluphosinate ammonium. The control plants, grown under the same conditions and treated with the same dose of herbicide, died within 15–20 days of the herbicide application. The expression of the *bar* gene was verified by electrophoretic studies where the 23 kD BAR enzyme was shown to be absent in the controls. Progeny obtained from selfed transgenic plants were able to germinate and develop in the presence of 10 mg/L PPT, indicating the transfer and expression of the gene in F_1 transgenic plants.

Key words: tobacco, *bar*, BASTA® resistance

Introduction

The transfer of bacterial genes coding for specific detoxification enzymes is a commonly used technique for the production of herbicide-resistant transgenic plants (Stalker, 1992; Streber and Willmitzer, 1989; Mazur and Falco, 1989; Lyon, 1993). Among these, the *bar* (Thompson, 1987) and *pat* (Strauch, 1988) genes isolated from the species *Streptomyces* are two commonly used genes in plant genetic engineering to develop transgenic crop plants that are resistant to broad spectrum herbicides based on gluphosinate, the ammonium salt of phosphinothricin (PPT).

D,L-PPT is the active ingredient of the herbicide BASTA®, where L-PPT is an inhibitor of glutamine synthase (E:C.6.3.1.1), a key enzyme for ammonia assimilation. The inhibition of glutamine synthase leads to ammonia accumulation, the impairment of photosynthesis and cell death in the plants (Tachibana, 1986). The *bar* and *pat* genes code with 85% identity for the polypeptides containing 183 amino acids (20.1 kD), refered to as BAR and PAT enzymes, respectively. Both enzymes are acetyltransferases that inactivate L-PPT by acetylating the free amino group of the molecule and show very similar structural and functional properties (Wehrmann, 1996).

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By using either gene under different chimeric constructions, numerous examples of plants for engineered the purpose of weed control can be found in the literature, including wheat (Vasil, 1992), maize (Fromm, 1990; Gordon-Kamm et al., 1990), rice (Datta, 1990; Rathore, 1993), barley (Wan and Leamaux, 1994) sugarbeet, potato, tomato, tobacco (De Block, 1987; Filho, 1994; Hall, 1996), pea (Grant, 1995) and *Brassica* species (De Block, 1995). In addition, these genes are widely used as selectable marker genes in transformation studies, especially with monocots (Wilmink, 1993).

The objective of the present work was to establish an *Agrobacterium*-based transformation system to engineer local varieties for the purpose of weed control via the transfer of the *bar* gene. The system was optimised in tobacco, and tomato, potato and lentil are currently under investigation.

Materials and methods

Plant material

2–3 -month-old tobacco (*Nicotiana tabaccum)* plants of the variety Samsun grown in pots were utilised in the experiments.

Bacterial strains and plasmids

The *Agrobacterium tumefaciens* strain LBA4404 carrying the pDHB321.1 binary vector (Fig. 1) was used throughout the entire study. This strain will be referred to as LBA:pDHB in the text. LBA:pDHB was propagated in LB medium supplemented with 25 mg/l streptomycin and 50 mg/l kanamycin.

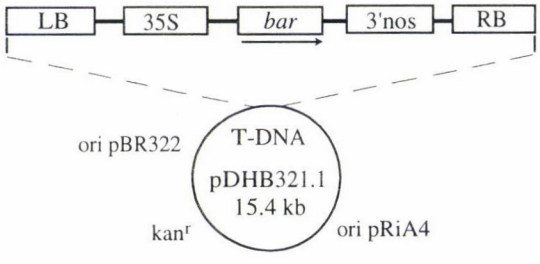

Fig. 1. General features and T-DNA region of binary vector pDHB321.1

Plant transformation and regeneration

The leaf disk transformation method of Horch et al., (1985) modified by Öktem (1994) was employed in the experiments. Leaves were surface sterilised for 10 minutes in 0.5% sodium hypochlorite and rinsed three times with sterile distilled water. Disks were prepared and treated with LBA:pDHB at OD_{600}:0.4–0.5 density, blotted to dryness and transferred to MS medium (Murashige and Skoog, 1962) supplemented with Gambor's B5 vitamins (Gamborg, 1968), 3% sucrose, 1 mg/l benzyl adenine (BA), 0.1 mg/l naphthalene acetic acid (NAA) and 0.7% agar, at pH 5.7 (MSA medium). Antibiotics and PPT were filter sterilised (0.2 μ) and added to the autoclaved medium. Following two days of co-cultivation, the disks were transferred to MSB selective medium (MSA supplemented with 10 mg/l PPT, 500 mg/l carbenicilin and 100 mg/l cefotaxime). The plates were refreshed at 3-week intervals. All the plates were kept in a controlled

environmental chamber at 25°C with 2000 lux illumination for 16 hours. In parallel experiments, leaf disks treated in the same manner except for the *Agrobacterium* infection were run as controls. Regenerated shoots were transferred to MSC root induction medium (MSB medium without BA and NAA). Finally, rooted plants were removed from the jars and transferred to pots with commercial soil.

Analysis of transformants

i, Leaf paint assay: The leaves of regenerated primary transgenic and control plants were painted with two different doses of BASTA® (1% and 3% v/v, equivalent to 2 and 6 g/L gluphosinate, respectively) before being transferred to pots. The evaluation was based on the appearance of necrosis on the leaf tissues.

ii, Herbicide application: Potted primary transgenic and control plants were sprayed with 1% (v/v) BASTA® preparation at a dose of 30 l/ha as recommended by the manufacturer (Hoechst). The evaluation involved a visual record of the appearance and growth behaviour of the plants, backed up with photographic documentation.

iii, SDS-PAGE: The product of the transferred *bar* gene, the BAR enzyme, was monitored electrophoretically. The total soluble proteins of leaf tissues of the primary transgenic and control plants were isolated according to Damerval et al. (1986). SDS-PAGE was performed according to Laemmli (1975). The gels were stained using Comassie Brilliant Blue.

iv, Analysis of F_1 progeny: Seeds obtained from selfed transgenic and control plants were surface sterilised (20% sodium hypochloride, 20 minutes) and transferred to MS basal medium (MSA without growth regulators) containing 10 mg/l PPT for germination. The ratio of germinated to non-germinated seeds was assessed 15 days later.

Results and discussion

Transformation, regeneration and selection

Callus formation was observed in the presence of 10 mg/l PPT, 3 weeks after co-cultivation, in leaf disks infected with LBA:pDHB. Almost 90% of the infected disks developed callus and shoots in the presence of 10 mg/l PPT. Although callus initiation was observed in some of the control plants in the presence of PPT, development stopped after 40 days of culture, followed by death at the end of 60 days of culture. Transformed disks formed numerous shoots after 40–60 days of culture. 70% of the shoots transferred to root induction medium containing 10 mg/l PPT were able to develop roots within a period of 40 days. After being potted, 60% of these plants died prior to herbicide application, indicating an overall transformation frequency of 25% in the given experimental system.

Analysis of primary transgenics

Leaf paint assay is a convenient method for measuring the herbicide tolerance of individually transformed plants prior to soil transfer, facilitating the selection and elimination of escapes prior to further analysis, thus saving time and labour. The primary transgenics tested were able to resist 1% and 3% (v/v) BASTA® applications (Fig. 2). After transfer to soil, the tested plants exhibited similar herbicide resistance behaviour where water application had no effect (Fig. 3).

Fig. 2. Leaf paint assay of primary transgenic and control plants.
Leaves of regenerated primary transgenic and control plants were treated with 1% and 3% (v/v) BASTA® preparations as described in Materials and methods. Jars were photographed after 9 days.

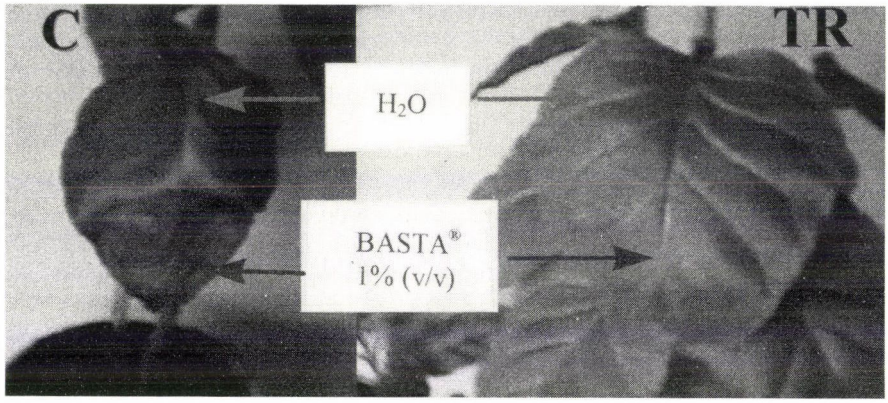

Fig. 3. Leaf paint assay of potted transgenic and control plants for *in vivo* herbicide resistance.
After 4 months of development in soil, the distal halves of randomly selected leaves of primary transgenic and control plants were painted with 1% (v/v) BASTA® and/or distilled water. Plants were photographed after 14 days. TR: Transgenic, C: Control.

Prior to herbicide application 50-60% of the plants died within 1 week following soil transfer. During the first month, primary transgenics exhibited slower development in pots compared to the non-transformed control plants. The primary transgenics tested survived a 30 l/ha, 1% (v/v) dose of BASTA®, equivalent to 2 g/l gluphosinate (Fig. 4).

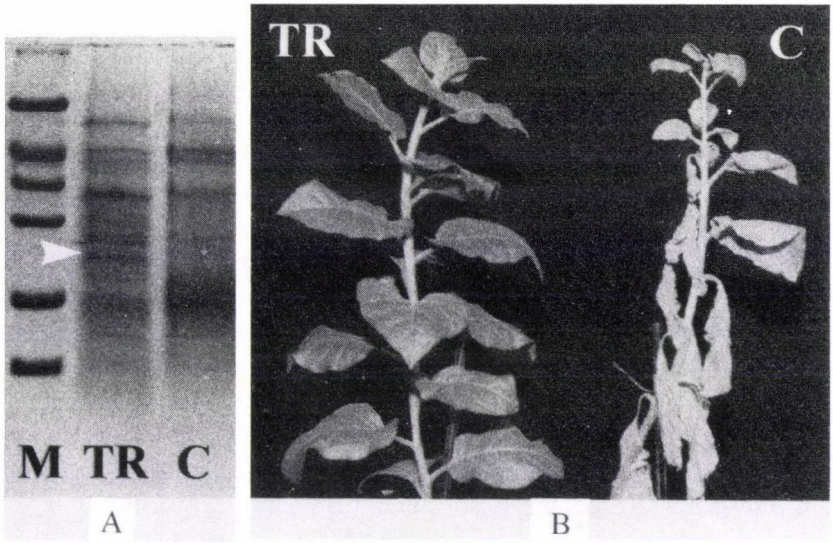

Fig. 4. Electrophoretic analysis and responses of primary transgenic and control tobacco plants to 1% (v/v) BASTA® application. **A)** Total soluble proteins extracted from the leaves of 3-month-old soil -grown primary transgenic and control tobacco plants were analysed by SDS-PAGE. Arrow indicates the location of the BAR protein. TR: Transgenic; C: Control, M: Sigma Molecular Weight Markers (Top to bottom: bovine serum albumin: 66 kD, egg albumin: 45 kD, glyceraldehyde 3-phosphate dehydrogenase: 36 kD, carbonic anhydrase: 29 kD, trypsin inhibitor 20.1 kD, α-lactalbumin: 14.2 kD). **B)** The same plants were sprayed with 1% (v/v) BASTA® and photographed after 14 days.

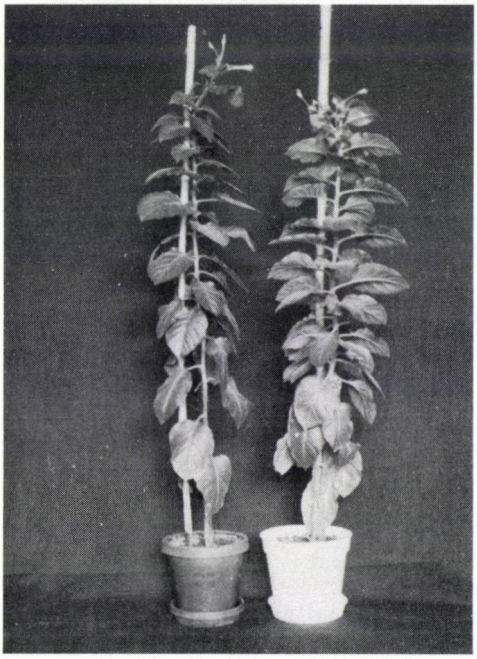

Fig. 5. Fully regenerated and seeded primary transgenic and control tobacco plants.
The primary transgenic plant that was resistant to 3% (v/v) ®BASTA treatment in the leaf paint assay (Fig. 1) was transferred to soil, self-pollinated and set seed. The photograph presents 7.5 months of development after soil transfer. The white pot demonstrates a non-transformed control plant at a similar development stage.

The molecular mass of the BAR enzyme was estimated to be 20.1 kD based on the amino acid sequence (Wehrmann, 1996). Previously it was shown that slightly higher molecular masses (22–23 kD) could be obtained in SDS-PAGE. In the present experiments, electrophoretic analysis of the proteins extracted from BASTA®-resistant plants exhibited a protein band of 22–23 kD that was absent in the control plants (Fig. 4). These results further confirmed the integration and expression of the *bar* gene in primary transgenics.

The primary transgenic plants that survived herbicide application grew to maturity and set seed (Fig. 5). The F_1 progeny obtained from selfed transgenic and control plants were also tested for herbicide resistance. None of the seeds obtained from the regenerated controls were able to germinate in the presence of PPT. However, the tested seeds of transgenic progeny survived lethal doses of PPT. Sample data are shown in Fig. 6.

Currently, experiments are underway for the development of PPT-resistant transgenic tomato, potato and lentil plants in local varieties.

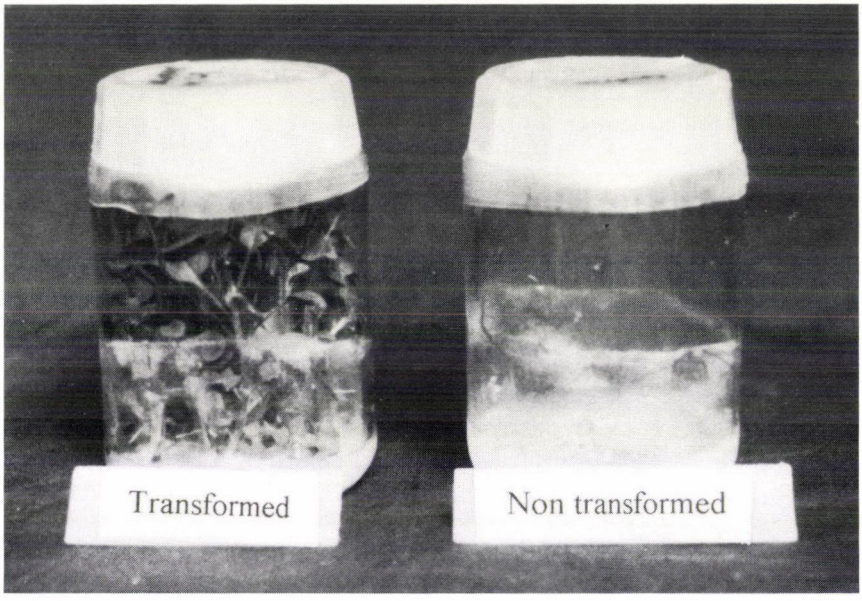

Fig. 6. Analysis of F_1 of progeny transgenic and control seeds in MS medium with 10 mg/l PPT. Seeds obtained from the plants shown in Figure 4 were surface sterilized and transferred to MS medium containing 10 mg/l PPT. The jars were photographed after 5 weeks.

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INTERACTION OF GENOTYPE, CULTURE MEDIUM, GROWTH REGULATORS AND LEVEL OF 2,4-D ON THE REGENERATION RESPONSE OF WHEAT

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The applicability of cereal tissue culture depends on reliable callus culture and plant regeneration procedures. The objective of this study was to investigate the interaction of different parameters, such as media, genotypes, the level of 2,4-D during callus induction and other growth regulators, on compact morphogenic callus production and regeneration ability as measured by shooting frequency and the number of shoots per regenerating callus. Immature embryo explants of eight different wheat genotypes were taken from 10–18-day-old spikes and cultured on two basal media, MS and B5, supplemented with 3 different levels of 2,4-D at 1.0, 1.5 and 2.0 mg/l. The callus induction frequency ranged from 51–81%, and the compact, nodular and morphogenic callus frequency ranged from 35 to 58%. Genotypes, media, growth regulators and the level of 2,4-D during callus induction were observed to have significant effects on the regeneration response. The medium has an important role in shoot induction ability as its interactions with genotypes, growth regulators and the level of 2,4-D and the medium × genotype × 2,4-D level interaction were significant. The genotype, on the other hand, was more important for the character shoots per regenerating callus. All the genotypes which showed good compact callus induction also exhibited high shoot induction and a larger number of shoots per regenerating callus. In some of the genotypes a shoot induction frequency of 90% and 5 shoots per callus could be obtained if the role of different factors was considered.

Key words: genotype, 2,4-D, growth regulators, interaction, regeneration, *Triticum aestivum*

Introduction

Cereal explants that contain immature meristematic cells develop callus which is competent to express totipotency. Regeneration has been achieved in wheat from various explants but immature embryos have been the most successfully utilized (Ahloowalia, 1982; Ozias-Akins and Vasil, 1982; Sears and Deckard, 1982; Chawla and Wenzel, 1987; Redway et al., 1990). Immature embryos are far more responsive than any other explant in culture. The success of regeneration in any crop depends upon the type of medium used in each phase of culture from callus initiation to maintenance and during regeneration. Media modified with different growth regulators have profound effects on callus induction and regeneration frequency. The auxin 2,4-D (2,4-dichlorophenoxy acetic acid) is routinely used for callus induction and its level in the medium affects callus induction and also has an indirect effect on regeneration

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(Atanassov et al., 1991). In addition, the concentration of auxins and cytokinins, and sometimes the synergistic effect of both, influences shoot induction ability. The present paper reports the individual and interactive effects of genotype, media, growth regulators and the level of 2,4-D during callus induction on the regeneration ability.

Materials and methods

The experimental material consisted of four timely-sown cultivars, CPAN 3004, WH 542, PBW 154 and UP 2003, and four late-sown cultivars, Sonalika, HD 2285, PBW 226 and UP 2121. Immature embryo explants measuring 1.0 to 1.5 mm in diameter were collected from the spikes 10–18 days after anthesis. These were surface sterilized with commercial sodium hypochlorite (1% active chlorine) for 10 min, followed by 3–4 washings with sterile distilled water. The immature embryos were cultured on two basal media, MS (Murashige and Skoog, 1962) and B5 (Gamborg et al., 1968). The sucrose concentrations in the MS and B5 media were 3% and 2% respectively, while standard concentrations of organic nutrients and vitamins were used. All the chemicals used were of analytical grade and the growth regulators were manufactured by Sigma, USA. The pH of the medium was kept at 5.7. The medium was solidified with 0.8% agar-agar (Hi-Media, India) and autoclaved at 121°C for 20 minutes at 15 p.s.i. The growth regulator zeatin was filter sterilized and added to the autoclaved medium, while all other growth regulators were co-autoclaved. Twenty ml of the medium was poured into 100 ml conical flasks. The basal media were supplemented with three different levels of 2,4-D growth regulator (1.0, 1.5, 2.0 mg/l) plus a control for callus induction. Five embryos per flask were inoculated with the scutellar axis facing up and the plumule-radicle axis in contact with the medium. After 4–5 weeks the induced calli were categorised as i) friable type: loose, watery and pale yellow to white in colour and ii) compact type: hard, nodular and yellowish in colour. The callus induced from each embryo of a genotype was maintained as a cell line on the basis of the 2,4-D concentration in the callus induction medium. The first subculture was made after 5 weeks on a medium containing the same level of 2,4-D and subsequently the callus was subcultured every 3 weeks by breaking the callus into pieces, being maintained on a basal MS or B5 medium with 1.5 mg/l 2,4-D. After at least 2 subcultures, the healthy-looking compact calli were inoculated on different regeneration media consisting of MS and B5 basal constituents modified with various growth regulators (Kin - kinetin, BAP - benzyl amino purine, NAA - naphthalene acetic acid) for shoot induction:

S0: Control

S1: 1.0 mg/l Kin + 0.2 mg/l NAA	S4 : 1.0 mg/l Kin
S2: 1.0 mg/l BAP + 0.2 mg/l NAA	S5: 1.0 mg/l BAP
S3: 1.0 mg/l Zeatin + 0.2 mg/l NAA	S6: 1.0 mg/l Zeatin

The cultures were kept in the dark at 25°C during callus induction and maintenance, while during regeneration the cultures were incubated at 25°C with a 16/8 h photoperiod and a light intensity of 3000 lux. The percentage shoot induction and the number of shoots per regenerating callus were recorded. Statistical analysis was conducted using a completely randomized design to study the significance of the various parameters.

Results and discussion

Callus could be induced from all the genotypes on both the basal media (MS and B5) when supplemented with 2,4-D. The mean callus induction frequency ranged from 51.5% in UP 2003 to 81% in PBW 154. The frequency

of callus induction in MS or B5 media at a 2,4-D rate of 1.5 mg/l was found to be in the range of 85–92% for the genotypes PBW 154, PBW 226, Sonalika and CPAN 3004. The callus obtained was either friable or of the compact nodular type. Compact callus has been reported in the literature to be morphogenic, so data were recorded on it. The MS medium produced 50.3% compact calli over all the genotypes as compared to 42.2% in the B5 medium. The genotypes PBW 226, PBW 154 and CPAN 3004 gave the highest compact callus induction in MS with a range of 57–67%. All the genotypes gave similar results in the B5 medium but with reduced frequency. With different levels of 2,4-D concentration in the medium, compact callus induction showed a frequency of 56.8% at 2 mg/l 2,4-D, 54.6% at 11.5 mg/l 2,4-D and 24.7% at 1 mg/l 2,4-D over all the genotypes and both the media. The medium × 2,4-D interaction over the genotypes revealed that the MS medium with 1.5 or 2 mg/l 2,4-D gave good callus with a frequency of 58–61% as compared to a 50–52% compact callus induction frequency in B5 medium at the same levels of 2,4-D. The auxin 2,4-D is the most common exogenous growth regulator added to cereal culture media. The results indicate that 1.5 to 2 mg/l 2,4-D is the optimum concentration for the production of embryogenic callus, while Redway et al. (1990) reported that 2 mg/l 2,4-D gave the highest formation of embryogenic callus. Caligari et al. (1987) also assessed the role of 2,4-D and its effect on the culture response of barley genotypes and found significant differences for the fresh and dry weight of callus.

Compact, hard, healthy-looking nodular calli were inoculated on different regeneration media and data were recorded on the percentage shoot induction and the number of shoots per callus. Analysis of variance for shoot induction and number of shoots per callus showed significant differences for genotypes, media and growth regulators (Table 1). The level of 2,4-D in the callus induction medium showed significant differences only for the shoot induction character. A peculiar pattern of interactions was observed if any interaction was significant for one character but non-significant for another character of regeneration response. A perusal of the table shows that the medium has an important role in shoot induction ability as its interactions with the other three factors (genotypes, growth regulator and level of 2,4-D) together and with genotype and the level of 2,4-D separately, were found to be significant. In the case of shoots per callus significant interactions were found when the genotype was involved in interactions with the level of 2,4-D, with medium × growth regulator and with medium × growth regulator × level of 2,4-D. Significant interactions were also recorded for medium × level of 2,4-D and growth regulator × medium × level of 2,4-D for shoots/callus.

The shoot induction frequency of different genotypes over all the media, growth regulator supplements and levels of 2,4-D in the callus induction media ranged from 63.5% in PBW 154 to 30% in UP 2003 and WH 542. The MS medium showed a significantly higher shooting frequency (52.1%) as compared to 33.5% in the B5 medium. The shooting frequency was 43.8%, 43% and 39.8% when the calli used for regeneration were initiated at 1.5, 1.0 and 2 mg/l 2,4-D in the callus induction medium, respectively . Media supplemented with zeatin and

Table 1
Significance of the main effects of genotype, medium, growth regulator and the level of 2,4-D during callus induction on the regeneration response of wheat according to analysis of variance and of their interactions

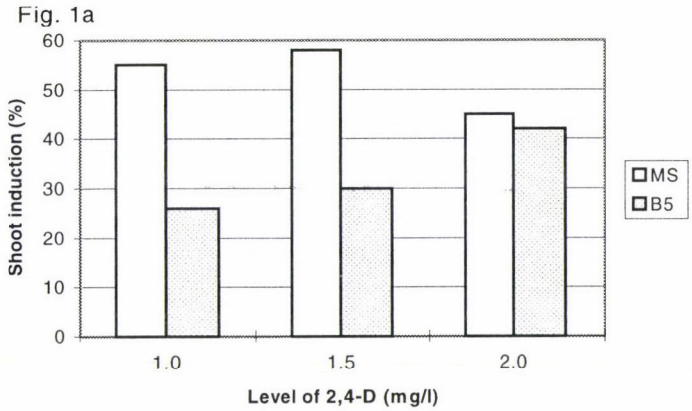
Source	d.f.	Average shoot induction	Shoots/callus
Genotype	7	**	**
Growth regulator	6	**	**
Medium	1	**	**
Level of 2,4-D	2	*	ns
Genotype × medium	7	*	ns
Growth regulator × medium	6	*	ns
Genotype × level of 2,4-D	14	ns	*
Medium × level of 2,4-D	2	**	*
Genotype × medium × growth regulator	42	ns	*
Genotype × medium × level of 2,4-D	14	*	ns
Growth regulator × medium × level of 1,4-D	12	ns	*
Genotype × medium × growth regulator × level of 2,4-D	84	ns	*
Error	84		

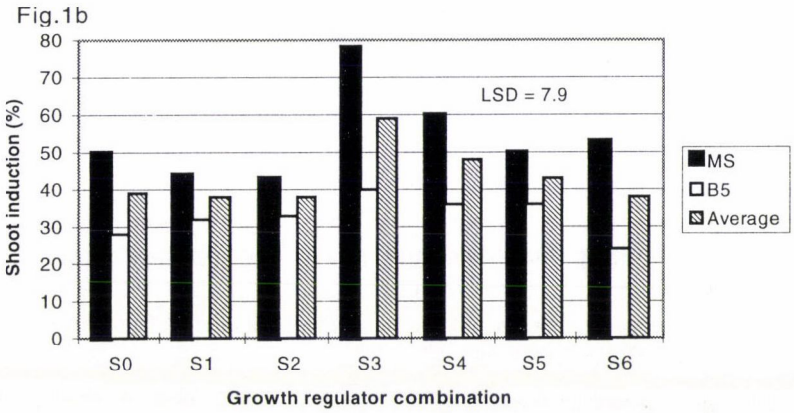
* and ** F-test significance at P = 0.05 and 0.01 respectively; n – non-significant

NAA gave the highest shoot induction (57.6%), whereas media without growth regulators showed 41% shoot induction ability (Fig. 2). Media supplemented with kinetin and BAP showed shoot induction rates of 47% and 43%, respectively. When these media were supplemented with NAA, the shooting frequency decreased by 2 to 6%, whereas media with zeatin gave improved shooting frequency when further supplemented with NAA.

The medium × level of 2,4-D interaction showed that callus induced in the MS medium with 1.0 and 1.5 mg/l 2,4-D resulted in 54 to 58% shoot induction, which was higher than the maximum obtained in the B5 medium (Fig. 1a). The medium × growth regulator interaction revealed significant differences. The best medium S3, containing zeatin and NAA, gave a shooting frequency of 57.6% averaged over the media. When this growth regulator combination was applied in MS basal medium it showed 76.4% shooting ability, while this figure was only 39% in B5 medium. This was the maximum shooting frequency obtained in B5 medium, but it was still lower than that recorded for any combination of growth regulator in the MS medium (Fig. 1b).

The medium × genotype interaction showed that shoot induction was greater on MS medium compared to B5 medium, with genotypes like PBW 154, PBW 226 and Sonalika showing a shoot induction of 70 to 71% in MS medium as compared to an average of 63–64% (Fig. 1c). On average, 10–15% more shoot induction was observed on MS medium as compared to B5 medium for each genotype. The importance of the genotype × medium interaction was emphasized by Carman et al. (1987), and it has now been proved beyond doubt that each genotype responds best in a particular nutritional milieu.





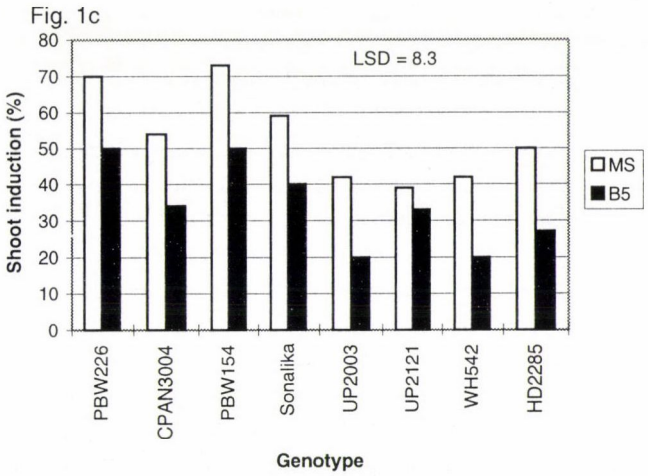


Fig. 1. Effect of different interactions on percentage shoot induction ability in wheat: a. Medium × 2,4-D level; b. Medium × growth regulator; c. Medium × genotype

The interaction between genotype × medium × level of 2,4-D in the callus induction medium showed that the shooting frequency can be increased to a maximum of 90.5% in the genotype PBW 226 when cultured on MS medium containing 2 mg/l 2,4-D. PBW 154 showed a maximum of 81% shoot induction in MS from callus induced at 1.5 mg/l 2,4-D and 85.7% in B5 medium with calli initiated at 2 mg/l 2,4-D. Thus, for a particular genotype, both the level of 2,4-D in the callus induction medium and the basal medium must be carefully chosen to obtain maximum shooting ability. In general, the genotypes showed high shoot induction percentages when the calli were induced on MS medium containing 1.0 or 1.5 mg/l 2,4-D or on B5 medium with 2 mg/l 2,4-D.

In different genotypes the shoots per callus character ranged from a value of 2.0 in PBW 154 to 0.7 in UP 2003 (Table 2). The MS medium showed an average of 1.6 shoots/callus, while B5 showed 0.8 shoots/callus. As observed for shoot induction ability, the growth regulator combination of zeatin with NAA gave the highest number of shoots/callus with an overall average of 1.6 shoots. The addition of the auxin NAA to media containing Kin led to a decrease in the number of shoots/callus, while an increase was observed for media containing BAP and zeatin supplemented with NAA. Medium-dependent responses have been reported in wheat by Sears and Deckard (1982) and Eapen and Rao (1985). Chawla and Wenzel (1987) reported that wheat responds better in MS medium and barley in B5 medium. It is evident that wheat requires a high concentration of inorganic salts for successful shoot regeneration compared to other cereals. The role of the genotype in regeneration ability has been reported by Wernicke and Milkovits (1986).

The overall effect of the level of 2,4-D on shoots/callus was non-significant, but its interaction with genotype was significant. All the genotypes except PBW 226 showed a greater number of shoots/callus when the callus induction medium contained 1.0 or 1.5 mg/l 2,4-D.

The genotype × medium × growth regulator interaction resulted in a maximum of 4.2 shoots/callus for PBW 226 in MS medium supplemented with zeatin and NAA, while if the level of 2,4-D was adjusted to 12.5 mg/l, 5 shoots/callus could be obtained (Table 2). The MS medium with zeatin and NAA proved to be the best combination, giving an average of 2.1 shoots/callus, followed by the BAP and NAA combination. The interaction between all the factors may result in a greater number of shoots for any genotype if the callus was induced on MS medium containing 1.5 mg/l 2,4-D and, in general, if the regeneration medium was supplemented with zeatin and NAA, though each genotype requires a specific combination to obtain maximum shoots/callus.

Breiman et al. (1987) and Papenfus and Carman (1987) reported that shoot induction from regenerable callus can be improved by transferring the callus to a medium containing cytokinin. Reducing the concentration of auxin (2,4-D) allows

Table 2
Effect of genotype × medium × growth regulator combination on number of shoots per callus in wheat

Genotype	Growth regulator combination							Mean
Medium	S0	S1	S2	S3	S4	S5	S6	
PBW 226								
MS	2.4	0.7	1.4	4.2	2.3	2.8	2.4	2.3
B5	2.7	0.6	0.4	0.8	1.0	0.7	0.8	1.0
Mean	2.5	0.7	0.9	2.8	1.6	1.7	1.6	1.7
CPAN 3004								
MS	2.0	1.4	1.8	1.6	1.3	0.3	0.3	1.6
B5	0.5	0.5	0.0	0.4	1.2	0.3	1.0	0.6
Mean	1.2	1.0	0.9	1.0	1.2	0.9	1.2	1.1
PBW 154								
MS	0.9	2.4	2.4	2.4	3.0	2.8	3.2	2.7
B5	1.2	0.4	0.4	2.6	1.2	2.8	1.7	1.5
Mean	1.0	1.4	1.4	2.8	2.0	2.8	2.4	2.0
Sonalika								
MS	2.0	1.3	2.3	1.9	1.4	0.8	1.5	1.6
B5	1.2	1.1	1.2	1.8	2.1	0.4	1.4	1.2
Mean	1.6	1.2	1.7	1.2	1.7	0.6	1.7	1.4
UP 2003								
MS	1.5	1.1	1.2	1.8	1.6	0.8	0.7	1.2
B5	0.0	0.0	0.0	0.0	0.6	1.0	0.2	0.3
Mean	0.7	0.5	0.6	0.6	1.1	0.9	0.4	0.7
UP 2121								
MS	0.6	1.1	1.5	2.2	0.8	1.9	1.7	1.4
B5	0.0	0.3	2.0	1.1	1.2	0.4	0.4	0.8
Mean	0.3	0.7	1.7	1.6	1.0	1.1	1.1	1.1
WH 542								
MS	0.5	1.0	1.5	1.8	1.0	0.8	1.1	1.1
B5	1.7	0.4	0.4	1.1	0.0	0.0	0.5	0.6
Mean	1.2	0.7	1.0	1.4	0.5	0.4	0.8	0.9
HD 2285								
MS	0.6	1.3	1.4	1.3	0.8	0.8	2.3	1.4
B5	0.6	0.5	1.5	0.4	0.0	0.0	0.6	0.8
Mean	0.6	0.9	1.5	0.8	0.4	0.4	1.4	1.2
MS	1.3	1.3	1.7	2.1	1.5	1.4	1.6	1.6
B5	1.0	0.5	0.7	1.0	0.9	0.7	0.8	0.8
Mean	1.2	0.9	1.2	1.6	1.2	1.1	1.2	1.2

Genotype × Medium × Growth regulator: CD – 0.6; SEM – 0.05

rapid embryogenesis or organogenesis (Wang and Nguyen, 1990). The endogenous auxin and cytokinin concentrations vary as post-anthesis zygotic embryos develop. Zeatin and zeatin riboside were found to be the dominant cytokinins at 3, 12 and 15 days post-anthesis (DPA) in maize. By contrast, zeatin levels go from almost undetectable to 180 µmols/l by 3–4 DPA and then

decrease rapidly during wheat embryo differentiation (Fellers et al., 1995). The addition of NAA and zeatin to the regeneration medium is an attempt to provide supplemental growth regulators. The results clearly showed that if a small quantity of auxin is added, especially in combination with zeatin, this increases the shooting frequency as well as the number of shoots/callus. In summary, the best results for each genotype must be identified by looking at various interactions, so that genotypes which are of commercial importance can be exploited in *in vitro* selection experiments against biotic and abiotic stresses and for genetic transformation.

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MULTIPLE ALLELISM AT THE *VRN1* LOCUS OF COMMON WHEAT

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Studies were made on near-isogenic lines of cv. Novosibirskaya 67 with *Vrn1* genes from different donor cultivars. The multiple allelism of the *Vrn1* genes was demonstrated in an allelism test. The dominant allele of Novosibirskaya 67 and the "weaker" dominant allele of Pirotrix 28 were designated *Vrn1a* and *Vrn1b,* respectively. The possibility of different alleles at the *Vrn1* locus controlling the variability of the vernalization treatment and the ear emergence time of common wheat is discussed in the paper

Key words: *Triticum aestivum*, growth habit, *Vrn* genes, multiple allelism

Introduction

The study of the genetics of growth habit (spring *vs.* winter) in common wheat *Triticum aestivum* L. started at the beginning of the century (Spillmen, 1909; Stoll, 1910). Five non-allelic dominant *Vrn* genes (responsive to vernalization) or combinations of them have been shown to determine spring growth habit [(for review see Flood and Halloran (1985), Rigin and Goncharov (1987)]. In the *Triticeae* the investigation of the multiple allelism of these genes determining spring growth habit has a long history. Hoffman (1944) was the first to suggest the possibility of multiple allelism and to connect each of the three alleles *S*, *s* and *s'* (from the German word Sommer) with spring, winter and intermediate growth habit, respectively, in barley. In the case of common wheat, multiple allelism at the loci determining growth habit was suggested by Tsunewaki and Jenkins (1961). Three alleles were proposed at the *Vrn1* locus by Klaimi and Qualset (1974), who designated the alleles as *D*, *d*, and *D'*. Snape et al. (1976), Roberts and MacDonald (1984) and Merezhko (1989) also implied the occurrence of multiple alleles at this locus. However, Efremova and Maystrenko (1996) maintained the opposite viewpoint. It should also be noted that none of the experiments cited above actually involved tests for allelism. The authors based their suggestions on the behaviour of cultivars or chromosome substitution lines, rather than test crosses.

The goal of this work was to obtain experimental evidence for the occurrence of multiple allelism at the *Vrn1* locus.

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Materials and methods

A pure line of the common spring wheat cultivar Novosibirskaya 67 (whose spring growth habit is controlled digenically by two dominant genes, *Vrn1* and *Vrn2*) and its BC_9 near-isogenic lines ANK-18A and ANK-20B, developed by Koval (1997), were used in the present work. The winter cultivar Bezostaya 1 was used as a donor of the recessive gene *vrn1* in the development of the line ANK-18A. The spring habit of this line is monogenic and is controlled by the dominant gene *Vrn2* originating from cv. Novosibirskaya 67 (Goncharov and Koval, 1988). The cultivar Pirotrix 28, bred at the Research Institute of Grain Farming (Shortandy, Kazakhstan), was used as the donor of the dominant gene *Vrn1* in developing the near-isogenic line ANK-20B. In this case the line ANK-18A was used as the recurrent parent, because it possessed the dominant gene *Vrn2* from cv. Novosibirskaya 67 and the recessive gene *vrn1* of cv. Bezostaya 1. Nine backcrosses were made, with selection being carried out for the earliest plants in each generation. Thus, Novosibirskaya 67 and the near-isogenic line ANK-20B both contain the same dominant gene *Vrn2* in a uniform genetic background but have different dominant *Vrn1* genes.

The response to vernalization was examined by exposing seedlings to a temperature of 0°C to +4°C for 40 days in a refrigerator. The significance of the difference between the ear emergence times of the plants was estimated by Student's t-test using conventional software.

The genetic control of the growth habit of the plant material employed was determined by hybridizing with the near-isogenic tester lines Triple Dirk D and Triple Dirk B, containing dominant genes *Vrn1* and *Vrn2*, respectively developed by Pugsley (1971), and with winter cv. Albidum 114. To identify the *Vrn* genotype F_2 segregation was scored according to the scheme published earlier (Dzhalpakova et al., 1996). Three months after sowing, when all the standard spring varieties had already headed, it was possible to classify the hybrid material into spring (ear emerged) and winter (no visible ear formation) types. The number of segregant plants in each cross combination was determined and was compared with the theoretical estimated segregation using the χ^2 method.

Results

Table 1 presents the results obtained when determining the genetic control of the growth habit of near-isogenic line ANK-20B and cultivars Novosibirskaya 67 and Pirotrix 28. In crosses between the cultivars and winter cv. Albidum 114 the hybrids segregated into two classes (spring vs. winter) according to a digenic scheme, but no segregation was observed in crosses between the cultivars and

Table 1
Identification of the *Vrn* constitution of genotypes of common wheat cultivars and near-isogenic lines

Cultivar or line	Segregation into winter *vs.* spring forms in the F_2 generation from crosses with			Genotype
	winter cultivar (*vrn1...vrn4*)*	Triple Dirk D (*Vrn1vrn2vrn3vrn4*)	Triple Dirk B (*vrn1Vrn2vrn3vrn4*)	
Novosibirskaya 67	192:13	483:0	369:0	*Vrn1Vrn2*
Pirotrix 28	270:12	152:0	880:0	*Vrn1Vrn2*
ANK-20B	148:90	131:0	178:0	*Vrn1Vrn2*

* Figures in brackets indicate the genotypes of the tester lines

either of Pugsley's near-isogenic test lines, Triple Dirk D (with the dominant gene *Vrn1*) and Triple Dirk B (with the dominant gene *Vrn2*). It is evident that the spring growth habit in both the cultivars studied and in the near-isogenic line ANK-20B is controlled digenically by the dominant genes *Vrn1* and *Vrn2*.

In all cases, both under field conditions (long photoperiods) and in the greenhouse during the autumn (short photoperiods), ear emergence in ANK-20B took place about 10 days later than in cv. Novosibirskaya 67. Following the vernalization of the seedlings this difference disappeared (Table 2). The difference between ANK-20B and cv. Novosibirskaya 67 was maintained under long and short daylengths, indicating that the differences could not be due to the gene *Ppd*, controlling the response to photoperiod.

Table 2

Days to ear emergence of unvernalized and vernalized common wheat cultivars and near-isogenic lines grown under an 18 h photoperiod in the greenhouse

Cultivar/Line	Days to ear emergence			Value of Student's t-test
	Unvernalized	Vernalized	Difference	
Novosibirskaya 67	44.9±0.75	45.9±0.39	1.0	0.90
ANK-18A	61.8±0.71	50.0±0.62	11.8	12.56*
ANK-20B	52.6±0.71	46.7±0.43	5.9	6.87*
Marquis	45.9±1.00	46.0±0.75	0.1	0.90

* significantly different at the 1% level

The results of the test for allelism are presented in Table 3. The backcross F_1 (ANK-20B × Novosibirskaya 67) × ANK-20B differed significantly from the backcross of Novosibirskaya 67 into the F_1 hybrid. Thus, Novosibirskaya 67 and ANK-20B carry different alleles of the dominant gene *Vrn1*.

Table 3

Allelism test between *Vrn1* growth habit genes from different donors analysed in the BC_1F_1 generation of hybrids between cv.Novosibirskaya 67 and ANK-20B

Cultivars/Hybrids	Genotypes	Number of plants	
		early ripening	late ripening
Novosibirskaya 67	*Vrn1aVrn1a Vrn2Vrn2*	18	0
(Novosibirskaya 67 × ANK-20B)	*Vrn1aVrn1b Vrn2Vrn2*	13	
× ANK-20B	*Vrn1bVrn1b Vrn2Vrn2*		15
F_1 Novosibirskaya 67 × ANK-20B	*Vrn1aVrn1b Vrn2Vrn2*	10	0
(Novosibirskaya 67 × ANK-20B)	*Vrn1aVrn1a Vrn2Vrn2*	18	0
× Novosibirskaya 67	*Vrn1bVrn1a Vrn2Vrn2*		
ANK-20B	*Vrn1bVrn1b Vrn2Vrn2*	0	20

Discussion

The interest of researchers in the question of the multiple allelism of the *Vrn* genes is connected with the fact that genes determining the duration of the vegetation period pleiotropically affect various agriculturally important characters, including yield (Stelmakh, 1993). In the long run, employing the multiple alleles of the dominant *Vrn* genes will increase the potential for manipulating the duration of the vegetation period. This is very important as all European wheat breeding has so far been confined to the use of combinations of only two dominant genes, *Vrn1* and *Vrn2* (Åkerman and MacKey, 1949; Stelmakh, 1990; Goncharov and Shitova, in press).

Klaimi and Qualset (1974) assumed the occurrence of three alleles of gene *D*, which they considered likely to represent gene *Sk* (presently designated as *Vrn1*). Law et al. (1976) postulated the occurrence of a series of multiple alleles at the *Vrn1* and *Vrn3* loci. Snape et al. (1976) surmised the existence of either three alleles of *Vrn1* or three separate genes on chromosome 5A, although these suggestions could not be confirmed by the experiments currently reported. The existence of multiple alleles at the *Vrn1* locus was also supported by Merezhko (1989). However, this hypothesis was based only on the differences between substitution lines and had not been checked through segregational analysis. However, the analysis of a series of 5A substitution lines, representing several cultivars, failed to reveal allelism at this locus (Efremova and Maystrenko, 1996).

Experimental verification of this hypothesis has been delayed because of the absence of suitable plant material. The isogenic lines created by one of the authors, having *Vrn1* genes substituted from different cultivars, made such an experiment possible.

Two catalogues produced by Stelmakh et al. (1987) and Rigin et al. (1985) list data on 756 and 177 common wheat cultivars, respectively. It was demonstrated that growth habit was determined by three dominant *Vrn* genes, of which only two are present in European cultivars. To explain all the differences between cultivars in the duration of the vegetation period, the above authors, similarly to Hoogendoorn (1985), were forced to ascribe part of the variability in days to heading as being due to genes controlling earliness *per se* (Stelmakh, 1981). The spring habits of Pirotrix 28 and the line ANK-20B, as well as that of cv. Novosibirskaya 67, are controlled by two dominant genes, *Vrn1* and *Vrn2* (Table 1). However, differences were found between them in the number of days to heading (Table 2). In the light of these data, it is inferred that the genetic system controlling spring growth habit is "more flexible" than was previously considered, and that the variability in the ear emergence time may be determined by different alleles at the *Vrn1* locus.

This result provides experimental support for the hypothesis of Rigin et al. (1985) that the difference between spring habit and intermediate (facultative spring) cultivars could be due to different alleles of the dominant gene *Vrn1*. This contradicts the generally accepted view of Pugsley (1971) that there is a single dominant *Vrn1* gene, epistatic to other vernalization genes, which shows little effect of dosage. Based upon the latter hypothesis, Stelmakh (1981) disagreed with Rigin's explanation of the intermediate growth habit in some cultivars. Stelmakh (1981) ascribed the genetic control of spring growth habit in intermediate cultivars exclusively to the dominant gene *Vrn2*, which possesses a weak phenotypic effect (the cultivars bearing this gene respond to vernalization by shortening the ear emergence time). Full verification of the hypotheses of Rigin or Stelmakh is beyond the scope of this study. However, the data obtained so far do support Rigin's hypothesis, although further additional experiments are needed to establish this beyond doubt.

In conclusion both the old and recently bred Russian cultivars of common wheat lack the weak allele of the dominant gene *Vrn1*, probably due to the climatic conditions of wheat cultivation. It would be reasonable to search Western European intermediate cultivars (those from Italy, Yugoslavia, for example) and tetraploid wheat species for this multiple allelism. Analysis of the pedigree of the cultivar Pirotrix 28, obtained through the crossing of common wheat cultivar Akmolinka 1 with the durum wheat cultivar Pseudogostianum 61 of unknown origin (Rabinovich, 1972), indicates that tetraploid wheat species could be a promising source. Akmolinka 1 was produced by crossing the Canadian spring cultivar Marquis with the winter cultivar Ukrainka ozimaya. The cultivar Marquis fails to respond to vernalization by shortening the time of ear emergence (Table 2). Thus, it is unlikely that this cultivar donated a variant allele of the dominant gene *Vrn1* to Pirotrix 28, suggesting that Pseudogontianum 61 could be its source.

Since we have provided direct evidence for two dominant alleles of the gene *Vrn1*, we suggest that the allele in the genotype of Novosibirskaya 67 be designated as *Vrn1a* and the weaker allele of Pirotrix 28 as *Vrn1b*.

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WATER LOSS AND MICROCLIMATE OF TWO MAIZE (*ZEA MAYS* L.) HYBRIDS

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A lysimeter experiment was conducted to evaluate modifications in evapotranspiration, microclimate and plant characteristics generated by two different maize hybrids at two N levels. Parallel with traditional measurements of evapotranspiration using lysimeters, some components of evaporation were also determined by micrometeorological techniques. Although the seasonal mean temperatures in 1996 and 1997 did not differ significantly, the temperature patterns were not the same. A cool spring in 1997 resulted in late sowing, so the growing season was 1 month shorter than in 1996. This explains the decrease in yield in 1997 compared with 1996 in each treatment. In both seasons the cumulative evapotranspiration (ET) of the hybrids with no N application differed significantly, in favour of MVK 480. The lower canopy resistance of MVK 480 confirmed the modification determined for ET. N use decreased the canopy temperature independently of the variety. The LAI values were about twice as high in 1997 as in the earlier season. The size of LAI influenced the soil surface temperature to the greatest extent. During the study, the microclimatic parameters varied according to both changes in LAI and stomatal resistance. At both N levels, the yield potential of MVK 480 was higher than that of Norma. Further investigations will be needed, especially at higher seasonal temperatures, to establish whether the apparent increase in MVK 480 yield persists in the case of unlimited water supplies.

Key words: evapotranspiration, LAI, stomatal resistance, temperature, yield

Introduction

The evaluation of evapotranspiration to provide a better understanding of the impact of different cropping practices on water use and plant microclimate is becoming increasingly important in both semi-arid and humid climates (Prueger et al., 1997). Methods of obtaining ET estimates cover a wide range, from direct measurements using lysimeters to methods based on the energy balance (Hatfield, 1990). In the present study a lysimeter was chosen, the use of which provides valuable information about temporal changes in water loss when a limited number of cropping systems are observed (two maize hybrids in this case). Micrometeorological techniques have increased in popularity because of recent improvements in sensor accuracy and due to the increased availability of portable equipment for field use. In addition to the lysimeters data collected using micrometeorological techniques were also used to improve the information on the artificially modified microclimate of the two different maize hybrids.

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The objective of the study was to describe the plant-water relationships of two different maize hybrids using unlimited water supplies and two N levels, with the emphasis on the altering plant parameters and microclimatic factors. Farmers can use these results to determine the economic implications when selecting the most appropriate maize hybrid for specific growing conditions.

Materials and methods

Lysimeter studies to measure differences in the plant-water relationship and yield of two different maize hybrids were carried out on the experimental area of the Agrometeorological Research Station in Keszthely during the 1996 and 1997 growing seasons. The lysimeter pots were filled with Ramann's brown forest soil with a mean bulk density of 1.5 Mg m^{-3} in the top 1 m of the profile and an available water capacity of 210 mm m^{-1}. This soil is characteristic of the surrounding area. Two maize hybrids were used which differed in their water demands. While the hydrid Norma SC (FAO 370) was water stress-tolerant, MVK 480 SC was bred for irrigated conditions. Both hybrids are commercially grown in Hungary.

The size of the experimental area was 0.7 ha and 16 compensation evapotranspirometer pots were installed along the southern edge of the field. In 1996 and 1997, seed was sown on 30 April and 15 May, and successfully emerged on 12 and 29 May, respectively. The reason for late sowing in 1997 was the cold dry spring. Plant density was 7.2 m^{-2} in both years. The only fertiliser N applied was 100 kg N ha^{-1} at planting to half the treatments. The other half (controls) were grown without N fertilisation. Two experimental factors were investigated in the study: variety, and nitrogen with unlimited water supplies. There were a total of 4 treatments with 4 replications:

– Norma control without N fertilisation (abbreviation: Norma O),
– MVK 480 control without N fertilisation (MVK O),
– Norma with 100 kg nitrogen ha^{-1} (Norma N),
– MVK 480 with 100 kg nitrogen ha^{-1} (MVK N).

Sixteen Thornthwaite compensation evapotranspirometer pots arranged in a complete block design were included in the study. For more details on the operation of the system see Anda (1986). The area surrounding the pots was irrigated daily to ensure a larger area for the microclimate measurements. Each pot had a 4 m^2 surface area, with a depth of 1 m. The pots were watered from below with unlimited supplies. Evapotranspiration values were given as the total daily water consumption of the pots, directly in mm.

Assimilatory surface size was characterised by weekly leaf area indices (LAI) measured with a portable leaf area meter of the LI-3000 type. The leaf area of the same 10 plants per treatment was measured throughout the growing season.

To measure stomatal resistance an AP 4 diffusion porometer of the Delta T type was used on a number of completely clear days in both seasons. Samples were taken randomly on the abaxial side of sunlit leaves of the same age and position on five representative plants within each treatment. The mean of five replications was used in the analysis of variance. The daily course of stomatal resistance was plotted using values measured hourly.

Micrometeorological instrumentation was located in the centre of the field. A schedule of ten 9- to 10-hour data collection periods was made to cover the whole measurement season after canopy closure. Air temperature and relative air humidity were registered at three levels (ground, cob and tassel level) with aspirated thermocouples connected to a LI-COR Model LI 1000-32 datalogger. The sensors were shaded to eliminate the effects of direct radiation.

Canopy and soil surface temperatures were sensed remotely on the days when stomatal resistance was measured. A Raytek II Model infrared thermometer with a 2° field of view and an

8 to 14 μm spectral band filter was used. The thermometer was fixed about 1 m above the canopy at an angle of 30° below the horizontal. The emissivities of plant and soil were assumed to be 0.96 (Fuchs and Tanner, 1966) and 0.90 (Anda, 1992), respectively. The average soil surface temperature was measured by walking along the rows for 60 s holding the instrument 0.1 m above the soil.

To measure the grain yield samples were taken after harvesting for dry matter estimation and were oven dried at 80°C to a constant weight.

All the data were analysed using combined analysis of variance across the years. Differences in means were compared using the LSD test.

Results and discussion

Weather conditions

The climatic conditions in the growing seasons investigated were not typical of the weather changes observed in the past decades (Fig. 1). The seasonal mean temperatures were 1.0°C and 0.8°C below the climatic norms in 1996 and 1997, respectively. The monthly mean temperatures of the periods investigated were very similar to each other: cool springs were followed by a fairly warm May and June. In the second half of the seasons the average air temperatures declined rapidly, reaching a maximum deviation of –2.4 and –4°C in the September of 1997 and 1996, respectively.

Unlike the air temperatures, the rainfall patterns of the two seasons differed significantly, especially in June and July, the most important period from the point of view of maize water requirements. Both mid-summer periods were anomalous with regard to their rainfall distribution, but the direction of deviation measured in 1996 was inconsistent with that determined for 1997. In 1997, it featured substantial, well-distributed amounts of rainfall. The sum of precipitation in June and July was 27.6 % higher than the respective 30-year means. In 1996 just the opposite was observed, the sum of rainfall in June and July was 31.8% lower than the 30-year average. Twice the normal rainfall was measured in September 1996, though this was of less importance for maize water demands.

Assimilatory surface size

Climatic conditions greatly influenced the green leaf area, and the seasonal differences always exceeded the influence of the different hybrids. The yearly averages of LAI in 1996 were approximately half ($P \leq 0.01$) the values recorded in 1997, having a maximum of 1.04–2.20 depending on the hybrid and the N treatment. In 1997, the LAI increased more intensively, reaching higher maximum values, with averages ranging between 1.42–3.87. This was probably due to the delay in sowing and the relatively warmer May and June in this year. In 1997 a rapid decline in the green leaf area of the control treatments was observed from the end of August (Fig. 2). As a result of nitrogen application the duration of the green leaves increased significantly ($P \leq 0.01$). In both seasons, independently of the hybrid, there was no significant effect on LAI when nitrogen was used.

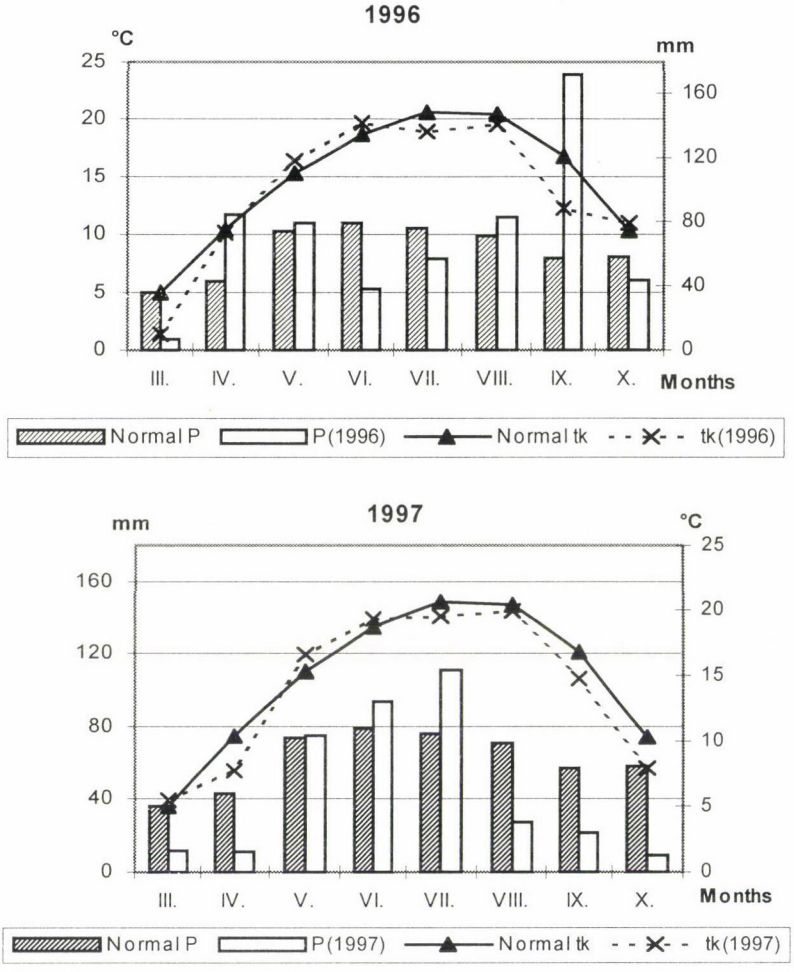

Fig. 1. Weather in the growing seasons investigated
(P = monthly precipitation sum, tk = monthly average temperature)

Norma was more sensitive to N fertilisation, and the increases in LAI were 84.4 and 92.3 % ($P \leq 0.01$) in 1996 and 1997, respectively. Over the same periods, the changes in LAI of MVK 480 were 70.4 and 53.1% ($P \leq 0.01$). In 1996 and 1997, the control plants of MVK 480 developed 21.5 and 45.2% greater assimilatory surface ($P \leq 0.01$) than those of Norma, making it possible for them to produce more photosynthates.

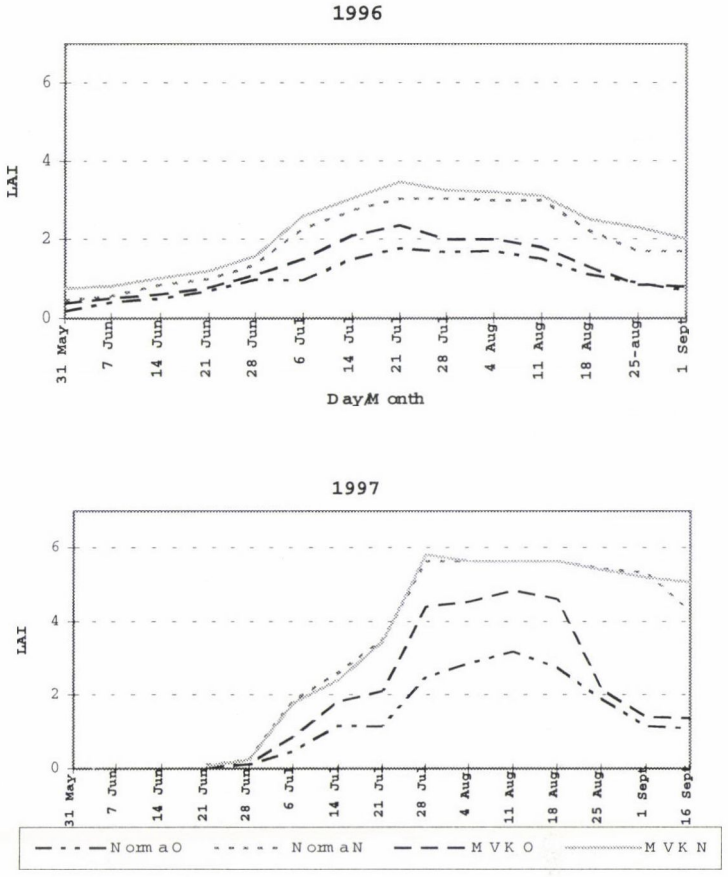

Fig. 2. Weekly leaf area indices in the 1996 and 1997 growing seasons

Evapotranspiration

Because lysimeters are known to be relatively slow systems, the seasonal evapotranspiration was characterised by pentad means together with cumulative ET curves (Fig. 3). The pentad means of ET at the same nutrition levels (N use and control) were seasonally consistent both in trend and values, because the monthly and seasonal temperatures were very similar during the two growing seasons investigated. At the beginning and end of the measuring periods there was no significant difference in water use in the different treatments. There was some deviation between the two seasons in the time when maximum water losses appeared. The highest daily water consumptions were observed a month later in 1997 compared to the peak water use of 1996, independently of the hybrid and nutrition level. In 1996 and 1997 nitrogen increased the water use of both Norma and MVK 480 by 21.9–29.0% ($P \leq 0.01$) and 15.1–26.9% ($P \leq 0.01$), respectively.

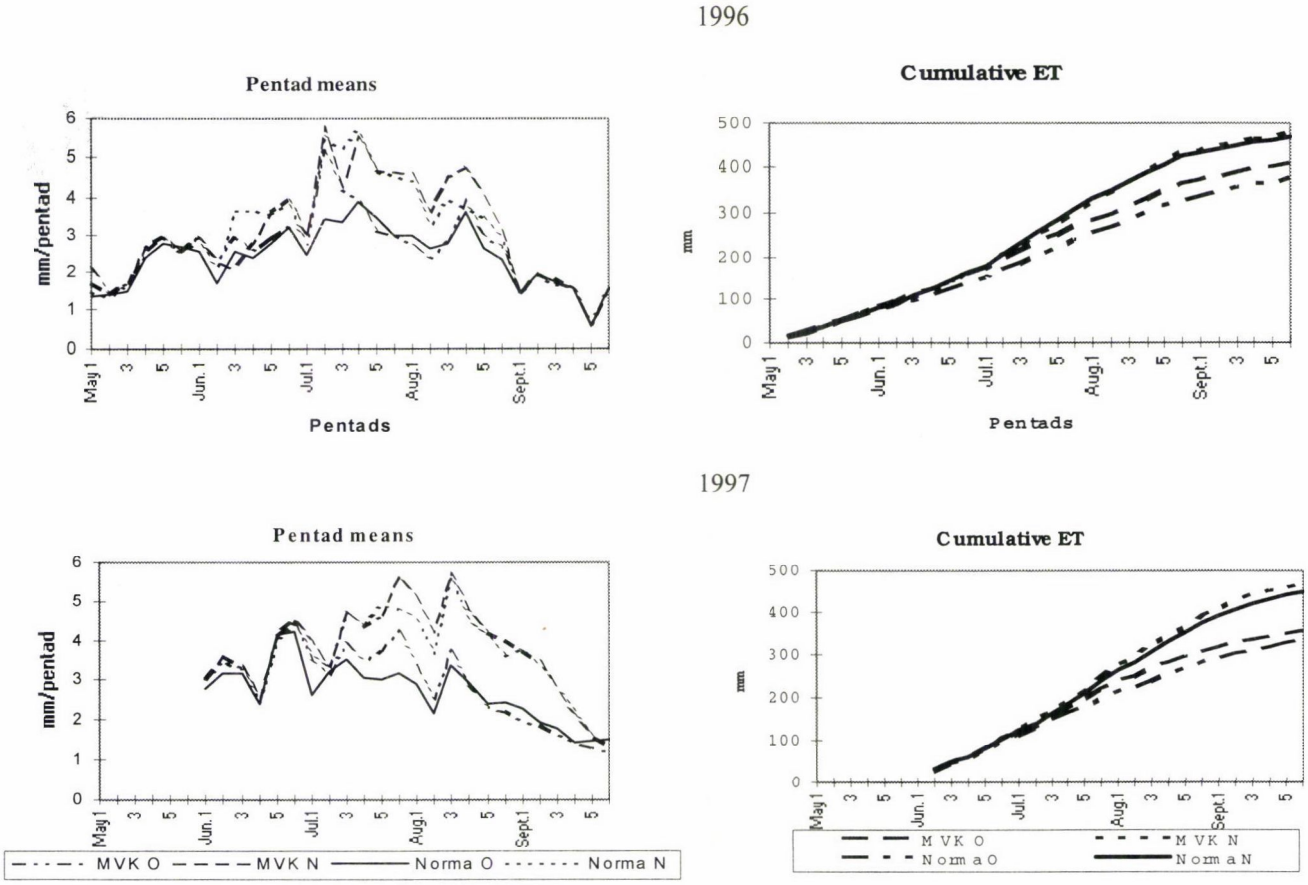

Fig. 3. Pentad means and cumulative evapotranspiration (ET) of the two maize hybrids

The effect of N on the seasonal water consumption of MVK 480 was more moderate than in the case of Norma. Significant differences between the cumulative ET values were only found between the two control treatments, and the water demands of MVK 480 were 8.6 and 6.1% higher ($P \leq 0.05$) than in Norma in 1996 and 1997, respectively. As the result of N application, the difference between the cumulative ET curves of the hybrids disappeared. Surprisingly, in the case of MVK 480, N application increased the LAI, but did not influence the cumulative water consumption of the hybrid. Differences in ET sums were only manifested in the no N treatments in both growing seasons, when the stomatal resistance altered significantly as well.

Stomatal resistance

In the present experiment the well-known seasonal tendency of stomatal resistance occurred: over the season the resistance decreased gradually until the middle of July, and later increased again. Although the changes resulting from the treatment showed the same tendency in both years, at the beginning and end of the measuring periods the actual daily means in 1996 were about twice as large as in 1997. In the middle of the seasons the daily average resistances were very similar to each other. As in the case of changes in cumulative ET, a significant difference between the two hybrids was observed in the control plots with no N application. The hybrid MVK 480 had 7.8 and 27.2% lower yearly mean stomatal resistance ($P \leq 0.05$) in 1997 and 1996, respectively, than Norma. The results are presented as the diurnal variation on sample days in each year (Fig. 4). The greatest significant changes were determined at low solar angles. In most cases, at high insolation the temporal variation in the stomatal resistance of the different treatments was moderated, and the high standard deviations meant that there was no significant difference between the treatments.

When N was applied the hourly resistances decreased significantly in both hybrids. In 1996 the mean stomatal resistance of MVK 480 dropped by 16.1% and that of Norma by 11.7% as the result of N application ($P \leq 0.05$). In 1997 these figures were 37.8% and 27.3%, respectively. In the control treatments the greater stomatal resistance of Norma might have been associated with the lower cumulative ET of the hybrid.

Canopy and soil temperatures

Because of the similar air temperature patterns of the two years investigated, there was little difference in the canopy temperatures. During the investigations the temperatures were very favourable for maize, since the maximum never exceeded the physiological limit value of 30°C. In fact the plant temperatures augured an advantageous season for maize in both years.

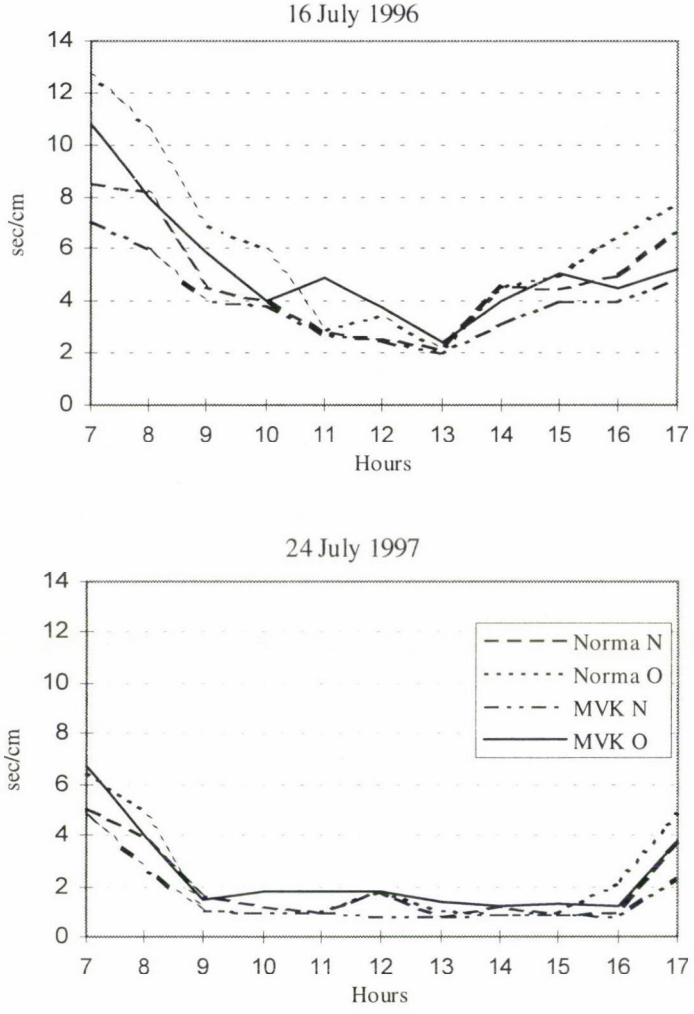

Fig. 4. Typical diurnal variation in the stomatal resistance of the maize hybrids

Depending on the time of observation, the daily mean temperatures of Norma were 0.8–2.1°C higher ($P \leq 0.05$) than those of MVK 480 during the two-year study. The largest differences were measured in mid-summer, especially in August 1996 and 1997 (Fig. 5). Independently of the hybrid, N use always decreased the canopy temperatures significantly. In each season the daily means in the no N treatments were 1.1–1.5°C and 1.6–2.5°C higher ($P \leq 0.01$) than in fertilised MVK 480 and Norma, respectively. Norma was more sensitive to changes in the N level.

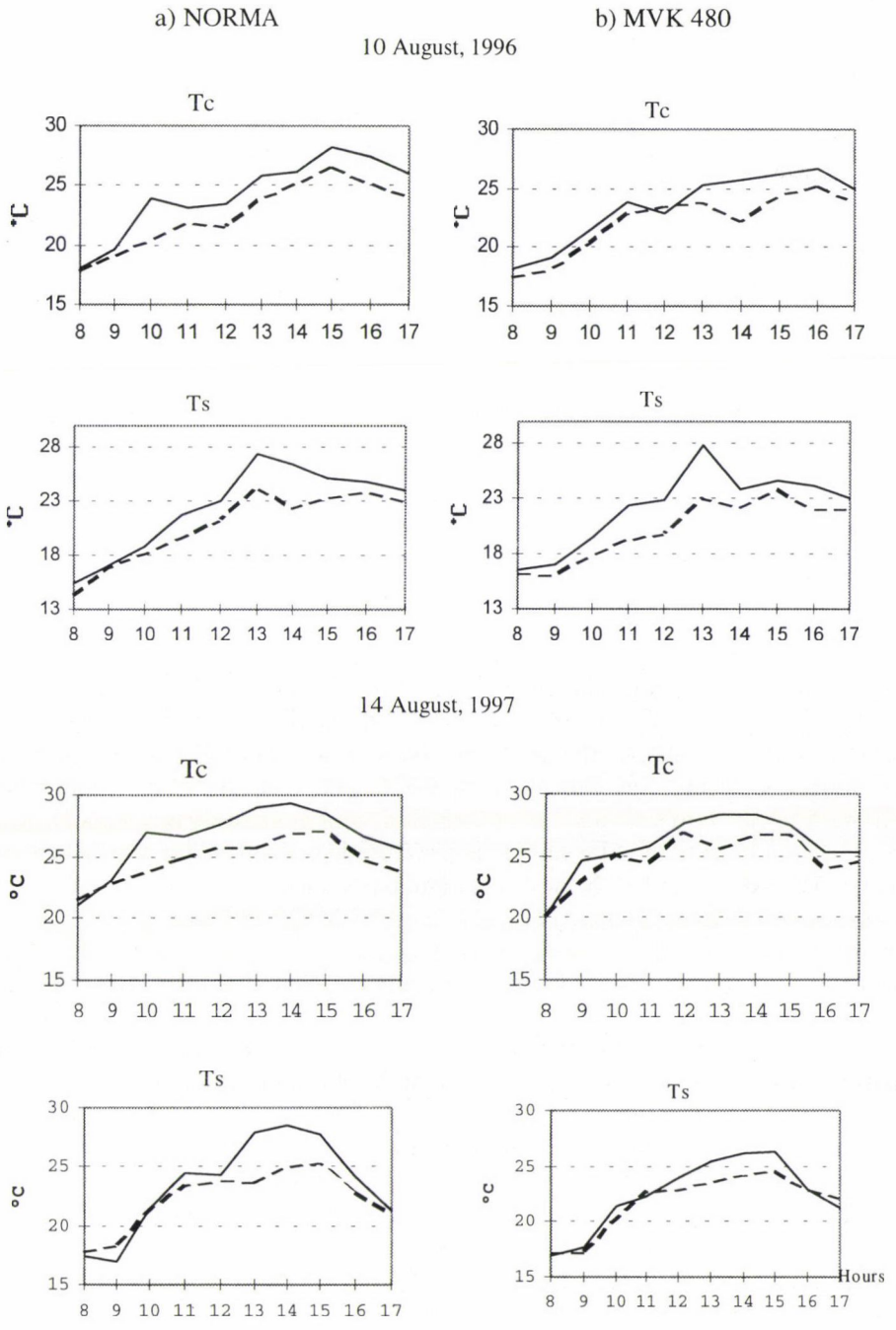

Fig. 5. Typical diurnal variation in crop (Tc) and soil temperature (Ts) in a) Norma and b) MVK 480 maize hybrids (control: continuous line; N use: broken line)

The higher the stomatal resistance, the higher the canopy temperature tended to be, irrespective of the hybrid. When the stomatal resistance is low, water cooling by transpiration is more effective, and causes a more moderate plant surface temperature. In both seasons the highest canopy temperatures were measured in July, but the greatest treatment differences were manifested in August (Fig. 5). Nevertheless, when N was applied the water losses of Norma did not differ significantly from the ET of MVK 480, though the canopy temperature altered. In the control treatments, the plant temperature relation of the hybrids was manifested in the evapotranspiration sums.

The soil temperature of the different treatments was under the control of the assimilatory surface size (Fig. 5). The greater the shading green leaf area, the lower the soil surface temperature. Before canopy closure the soil surface temperatures were very similar in all treatments. From the middle of July, the soil of MVK 480 treated with N was the coolest of all. A moderate but non-significant increase of 0.4–0.8°C in the daily means of Norma was determined in both years after N application. The Norma control, which had the smallest LAI, was the warmest treatment of all. The daily means of MVK 480 were 0.6–1.5°C less than the means of Norma in the control treatments ($P \leq 0.05$).

Microclimate of hybrids

The parameters of the plant microclimate combine the influence of the shading leaf area and other plant features that modify the radiation and water balance of the canopy. As the difference betwen the microclimate parameters of the four treatments was the same in the 2 years of the study, one typical variation for air temperature and humidity content at three different plant height levels at low and high solar angles is presented in Fig. 6. The higher leaf area size of MVK 480 shaded the soil more intensively and caused lower soil surface temperatures than in Norma. The cooler soil of MVK 480 was able to pass less energy to heat the air of the plant stand. Because of the more open canopy structure, the air of the control treatments in both hybrids was 1.6–3.2°C warmer than the air of the N-fertilised plant stands. Over the seasons at both solar angles there was less temporal variation in air temperatures between the maize hybrids investigated (0.3–1.5°C) than between the soil temperatures.

The relative humidity contents of the different treatments followed the changes in air temperatures. As the water-holding capacity of the air depends on the temperature, the warmer the air, the lower the relative humidity content (Fig. 6). There was a 6–24% difference over time in the relative humidity content of the air in favour of the hybrid MVK 480. N application increased the transpiration leaf area, thus also increasing the humidity content of the air by about 5–10%, irrespective of the hybrid.

Microclimate parameters measured at different plant heights exhibited random changes.

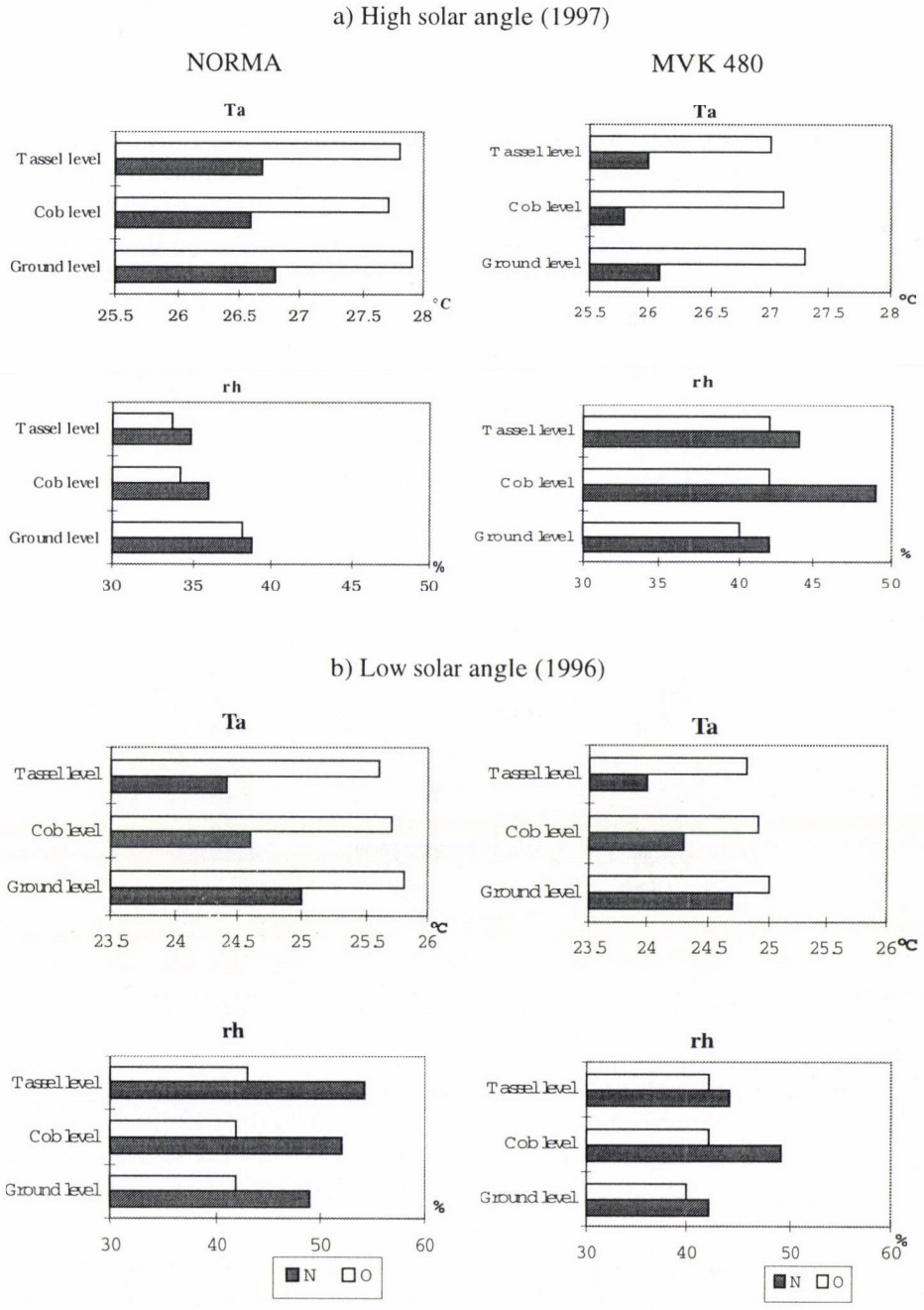

Fig. 6. Representative profiles of air temperature (Ta) and relative humidity (rh) at a) high and b) low solar angles in the first ten days of August 1996 and 1997

Yield of maize

In both years maize yields varied according to both the treatment and the length of the entire growing season (Fig. 7). The one month longer vegetation period and the relatively low air temperatures (together with even monthly rainfall distribution) in 1996 were more favourable for maize than the growing conditions (cool spring in March and April and late sowing) in 1997. In the controls the grain yields in 1997 were about half those obtained in 1996. This was surprising, as it meant that the twice higher LAI in 1997 was unable to produce a greater grain yield in either hybrid. The extra biomass production due to the increased assimilatory surface led to taller plants (approximately 0.25–0.45 m difference in the final heights of the hybrids). In spite of the approximately double leaf areas in 1997, the 1 month shorter growing season appears to have caused a deterioration in the generative development of the hybrids. Similar results were obtained in treatments with N application.

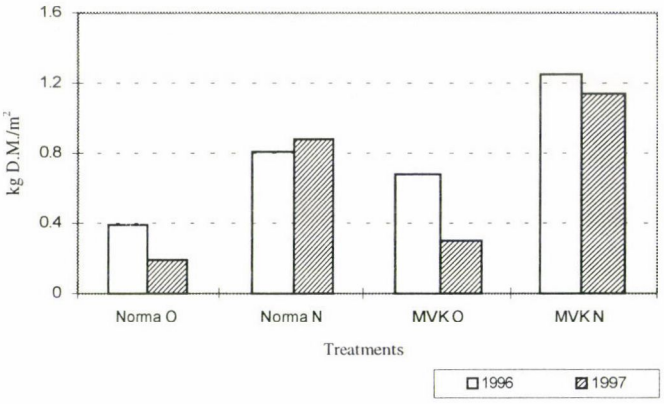

Fig. 7. Dry matter (D.M.) yield of the hybrids per unit area

The yield potential of MVK 480 was consistent at both nutrition levels. Without N fertilisation the yield surpluses of MVK 480 over Norma were 75.3 and 50.0% ($P \leq 0.01$) in 1996 and 1997, respectively. When N was applied the advantage of MVK 480 dropped by about half, being 42.7 and 25.7% ($P \leq 0.01$) in 1996 and 1997.

Nitrogen nutrition was relatively more effective in Norma than in MVK 480. While the yield surpluses in MVK 480 were 59.1 and 116.7% ($P \leq 0.01$) in 1996 and 1997, respectively, the results for Norma were 70.0 and 132.1% ($P \leq 0.01$).

Conclusions

During the 2 years of the experiment the weather was notably cooler and wetter than the 30-year average in Keszthely. In the 1997 growing season the relative humidity of the air at the solar noon only dropped below 45% four times, a phenomenon which has never been observed before. The air temperatures in the two growing seasons were very close to each other. For this reason neither the temporal changes in evapotranspiration nor the cumulative water loss of the two hybrids changed significantly when using 100 kg/ha N doses. Differences in the water consumption of the hybrids could be observed in the control treatments, where MVK 480 had higher water demand and lower stomatal resistance than Norma. As a result of N application, the differences in the annual water consumption of the hybrids disappeared.

In 1997 the LAI of the maize was twice as high as in 1996. This change did not result in more yield, but in taller plants. The length of the growing season was 1 month shorter in 1997 than in 1996. This may have caused disturbances in generative development, thus leading to the yield losses observed.

The greater shading leaf area of MVK 480 reduced all temperatures (soil, canopy and air). The cooler air temperatures, together with higher transpiration intensities due to the lower stomatal resistance of the plant stands, caused an increase in the relative humidity content of the air. In the present investigation, N use always moderated the differences in microclimate parameters between the hybrids.

Norma is a water stress-tolerant hybrid that was selected for dry weather conditions. When water supplies are unlimited (ET pots), or in the field when precipitation is close to or above the climatic norm, its yield potential is much worse than that of MVK 480. Norma is recommended for cultivation on areas where water is the limiting factor in most years.

Since the weather, especially the air temperatures throughout the growing seasons, was not representative of the past decades, the investigations will be continued. The behaviour of the hybrids may be significantly different in warmer growing seasons.

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RESULTS OF DURUM WHEAT BREEDING IN MARTONVÁSÁR

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Work on durum wheat (*Triticum durum* Desf.) has been in progress in Hungary, with varying degrees of success, since the beginning of the century. At first this work mainly involved basic research, followed later by introduction, breeding and cultivation. Its sowing area has fluctuated, and so far it has never been grown on more than 14 thousand hectares (1996). This slow spread can be explained chiefly by the fact that spring varieties were first grown, as no real winter varieties were available, and the productivity of these was far lower than that of winter wheats. Things began to change when the first winter durum wheat varieties were developed, but these still left much to be desired (poorer productivity, frost resistance and quality). Research was thus aimed at overcoming these deficiencies.

As the result of continuous efforts in the Agricultural Research Institute of the Hungarian Academy of Sciences in Martonvásár two varieties bred in Martonvásár (Martondur 1, Martondur 2) and two introduced varieties (Odmadur 1, Odmadur 2) were granted state registration in 1996, thus improving the choice available to growers and providing varieties better suited to their requirements.

All four varieties comply with DUS regulations. They are *productive*, yielding more in comparative state trials than the mean for the control varieties. In eight years of experiments in Martonvásár they gave yields similar to that of Yubileinaya 50. They have excellent *frost resistance*, approaching that of Bánkúti 1201, the common wheat variety grown in Hungary for the longest period (1931–1972); the only exception to this is Martondur 2, although even this has frost resistance equal to that of winter barleys. They tiller well and have good regeneration ability. They are mid-early varieties and are not prone to seed shrivelling under normal climatic conditions. Martondur 1 and Odmadur 1 have short stems, strong straw and good lodging resistance, while the other two varieties may lodge under unfavourable conditions. They have average resistance to the major diseases. When grown with satisfactory technology the quality of all the registered varieties reaches that laid down in the standard.

Key words: winter durum wheat, yield, quality, frost resistance, disease resistance, breeding, new varieties

Introduction

Some 90% of the wheat-growing area of the world is sown with varieties of the species common wheat, *Triticum aestivum* L., while durum wheat (*Triticum durum* Desf.) is grown on 8–10% of the sowing area (Koltay and Balla, 1982), thus making it the wheat species grown on the second largest area in the world (Szunics, 1986). Veneny (1939) considers the lower spread of durum wheat to be due to its poorer adaptability and lower productivity.

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According to Beke (1980) this species is grown on a total area of 20–30 million hectares, fluctuations in which are due to changes in the consumption of pasta, which has become more popular recently. In Europe it is grown on 2.1–2.2 million hectares, 1.7 million of which is to be found in Italy, where more than half the wheat-growing area is sown with durum wheat (Fabriani and Lintas, 1988).

In Hungary attempts at popularising durum wheat were made in the first decades of this century (Grábner, 1916; Odry, 1929; Havass, 1930; Veneny, 1939) and again in the seventies (Erdei, 1976; Beke and Barabás, 1981; Szániel, 1982). Szabó (1981) was of the opinion that a sowing area of 25–30 thousand hectares would be sufficient to supply the needs of the Hungarian pasta industry, and it was hoped to achieve this aim by the end of 1985. However, despite the fact that the sowing area of winter durum wheat varieties in Hungary is increasing, this figure has still not been reached. The sowing area increased from 225 hectares in 1980 to 9600 ha in 1989, but this was followed by a sudden drop to 960 ha in 1991. Since then a steep rise has been seen again and it is estimated that durum seed was sown on 14 thousand hectares in 1996. Depending on the year, yield averages have ranged from 2.9 to 4.5 t/ha (Beke and Matuz, 1996; Szunics, 1997). Economic factors are likely to play an important role in further increases in growing area and yield average, though neither the climatic and growing conditions (Erdei and Gyenes, 1981) nor the agronomic properties of the varieties grown (Szunics et al., 1985) can be ignored.

The primary reason for all the efforts that have been made to introduce, breed and cultivate *T. durum* is the excellent pasta-making quality of the species. It produces a higher yield of semolina, which can be used to make yellow-coloured pasta with good cooking value and favourable organoleptic quality (flavour, smell and texture) without the use of eggs or other additives. Pasta made without eggs is advantageous from the hygiene and storage point of view.

The cultivation of durum wheat varieties in Hungary is motivated by the following factors (Szunics and Szunics, 1992):

- the climatic conditions are more or less satisfactory,
- the demand for high quality pasta is increasing,
- it would enable the raw materials for pasta-making to be supplied without the need for imports,
- it could be an alternative crop to bread wheat,
- it would open up a new export market.

The slow spread of this crop can be attributed chiefly to the fact that to start with, as there were no true winter varieties, attempts were made to grow spring varieties, the productivity of which was considerably poorer than that of winter wheats. Things began to change when the first winter durum wheat varieties were developed, but these still left much to be desired (poorer productivity, frost resistance and quality). Research in Hungary was thus aimed at overcoming these deficiencies.

Experiments on durum wheat have been underway in the Agricultural Research Institute of the Hungarian Academy of Sciences in Martonvásár since the 1950s, shortly after its foundation. At first this work mainly involved basic research, while later the possibility of practical application was also investigated. When attempting to bring more variety into the basic stock used in *Triticale* breeding, Kiss (1953) made use of *T. durum*. Rajki (1961) studied the length of the vernalisation period. Numerous foreign varieties were sown in Hungarian experiments, leading to the publication of valuable data on yield potential (Szunics et al., 1985; Szunics and Szunics, 1992), quality (Pollhamerné, 1981), frost resistance and winter hardiness (Beke and Sutka, 1983; Szunics et al., 1985) and disease resistance (Szunics et al., 1997).

Since the 1982/83 season this work has gained new impetus. Wide-ranging international connections have made it possible to collect a large number of winter durum genotypes developed abroad, the agronomic traits of which have been tested for two fundamental purposes:

1. introduction, if any of the foreign varieties have favourable economic traits which satisfy the requirements of growers and pasta manufacturers;
2. breeding (variety development): each year 30–40 new hybrid combinations are created from Hungarian and foreign stocks. The genotypes best suited to Hungarian climatic conditions are selected from the segregating populations. The quality traits of the progeny are then evaluated in various phases of the breeding process.

It was hoped to achieve the following breeding aims:

1. improved productivity to at least the yield potential of moderately productive bread wheats;
2. better adaptability;
3. improved yield stability
   - better frost resistance and winter hardiness, at least equal to that of winter barley;
   - resistance to the diseases causing the greatest damage in Hungary (powdery mildew, leaf and stem rust, spike fusarium, leaf spot, black point, barley yellow dwarf virus, wheat dwarf virus);
   - improved lodging resistance;
4. a quality level satisfying Hungarian and international requirements.

For this purpose comparative field experiments were set up to determine the productivity and other agronomic indices of various genotypes, while their disease resistance was tested in the artificial infection nursery. Frost resistance is tested under artificial conditions in the phytotron, while major quality parameters are analysed in the laboratory. It is thus possible to develop productive, winter-hardy varieties with good quality and resistance to diseases.

After testing numerous foreign varieties, two lines of Ukrainian origin were found, which were registered in 1996 under the names Odmadur 1 (Hordeiforme 1443–82) and Odmadur 2 (Leucurum 1362–83). Two varieties

bred in Martonvásár were also registered in 1996, Martondur 1 (MvTD 28–94) and Martondur 2 (MvTD 32–94). This improved the choice available to growers and provided varieties better suited to their requirements. All four varieties comply with DUS regulations.

Materials and methods

The work was carried out in the experimental nurseries, laboratories, greenhouses and phytotron of the Agricultural Research Institute of the Hungarian Academy of Sciences, Martonvásár from 1983 to 1997. In the field experiments the wheat was sown after peas each year in carefully prepared soil at the optimum date. The yield potential and major agronomic traits of the varieties were determined in a random block design with 4 replications, on plots measuring 8 m^2. Disease resistance was evaluated on a 0 (resistant) to 9 (susceptible) scale. Viruses were diagnosed using the *Elisa* test. Diagnostic materials manufactured by *Agdia* and *Sanofi* were used to identify lines of the barley yellow dwarf virus and wheat dwarf virus, respectively (Pocsai et al., 1997).

Quality parameters were determined using the methods laid down in the standards. Details of the methods applied in the frost resistance and winter hardiness experiments were reported by Veisz and Rajki (1984) and Tischner et al. (1997).

In the various experiments the varieties Yubileinaya 50, Bánkúti 1201 *(T. aestivum)*, GK Basa, GK Tiszadur (*T. durum*) and Kompolti Korai (*Hordeum vulgare*) were used as controls.

Results and discussion

Yields of winter durum wheat varieties

The yield data achieved in Martonvásár from 1983 to 1997 are presented in Table 1. The control variety Yubileinaya 50 (*T. aestivum*), which was registered due to its excellent properties in 1970, was grown on the largest area in 1977 (27.1%) and is still cultivated. Over the thirteen years of the experiment this variety gave an average yield of 6.70 t/ha, with the highest yield in 1994 (8.65 t/ha) and the lowest in 1993 (5.55 t/ha), the difference being 3.1 t/ha. The variety GK Basa, bred in Szeged and registered in Hungary in 1980, and the variety Parus, originating from Odessa (Ukraine) yielded 15.94% and 10.65%, respectively, less than the control variety. In the best years these varieties approached or even exceeded the yield of the control (GK Basa, 1994: 113.06%; Parus, 1990: 109.09%), but there were also years when they yielded only half or less than the control (GK Basa, 1993: 44.50%; Parus, 1996: 29.47%). The yield fluctuations of these two durum varieties were thus considerably larger (GK Basa: 7.31 t/ha; Parus: 4.77 t/ha) than that of the bread wheat (3.1 t/ha), drawing attention to the greater genetic vulnerability of durum wheat varieties and their more intense sensitivity to technological and ecological factors. If the data of the individual years are compared, substantial differences can be observed between the varieties. In 1997, for example, GK Basa yielded 112.19% compared to Yubileinaya 50 and Parus only 94.94%, while in 1990 the two varieties gave the opposite performance. If the 1985 yield data, are considered, on the other hand, their yields were much the same, being 54.67 and 53.89% of the control, respectively.

Table 1
Grain yields of winter durum wheat varieties (Martonvásár, 1983–1997)

Year	Yubileinaya 50		GK Basa		Parus		GK Tiszadur		Odmadur 1		Odmadur 2		Martondur 1		Martondur 2	
	t/ha	%	t/ha	%	t/ha	%	t/ha	%	t/ha	%	t/ha	%	t/ha	%	t/ha	%
1983	7.58	100.00	6.01	79.29	7.54	99.47										
1984	7.01	100.00	7.18	102.43	6.84	97.57										
1985	5.14	100.00	2.81	54.67	2.77	53.89										
1988	5.78	100.00	5.01	86.68	4.77	82.53										
1989	5.81	100.00	4.94	85.03	5.31	91.39										
1990	6.05	100.00	3.82	63.14	6.60	109.09	4.66	77.02	6.18	102.15	7.24	119.67				
1991	6.60	100.00	6.84	103.64	6.58	99.70	4.01	60.76	9.11	138.03	7.65	115.91				
1992	7.32	100.00	5.83	79.64	6.80	92.90	4.36	59.56	6.74	92.08	6.40	87.43	6.90	94.26	7.66	104.64
1993	5.55	100.00	2.47	44.50	4.97	89.95	3.82	68.83	5.76	103.78	4.91	88.47	4.82	86.85	4.61	83.06
1994	8.65	100.00	9.78	113.06	8.67	100.23	9.62	111.21	9.87	114.10	9.05	104.62	11.33	130.98	10.28	118.84
1995	7.66	100.00	6.60	86.16	7.83	102.22	6.57	85.77	8.12	106.01	7.75	101.17	8.37	109.27	7.88	102.87
1996	6.21	100.00	3.25	52.33	1.83	29.47	5.06	81.48	2.31	37.20	3.33	53.62	2.17	34.94	4.75	76.49
1997	7.71	100.00	8.65	112.19	7.32	94.94	7.96	103.24	7.80	101.17	8.15	105.71	9.22	119.58	8.57	111.15
Mean																
1983–1997	6.70	100.00	5.63	84.06	5.99	89.39										
1990–1997	6.97	100.00	5.91	84.74	6.33	90.76	5.76	82.62	6.99	100.25	6.81	97.72				
1992–1997	7.18	100.00	6.10	84.87	6.24	86.84	6.23	86.75	6.77	94.20	6.60	91.86	7.14	99.33	7.29	101.51

Three further winter durum wheat varieties were included in the experiments from 1990, the results of which are also shown in Table 1. Over the eight years from 1990 to 1997 GK Tiszadur (one of the official state control varieties in durum experiments) averaged 5.76 t/ha, i.e. 17.38% less than Yubileinaya 50. The difference between the highest (9.62 t/ha) and lowest (3.82 t/ha) yields was 5.80 t/ha. Yield data are also available from this year for the introduced varieties Odmadur 1 and Odmadur 2, which had yield averages similar to that of Yubileinaya 50, averaged over the eight years. This does not mean, however, that there was no difference between the varieties. Odmadur 1 responded with outstanding yields to favourable climatic and technological conditions, while there were intense yield losses when conditions were unfavourable. The difference between the highest (9.87 t/ha) and lowest (2.31 t/ha) yields was consequently 7.56 t/ha. This difference was almost two tons less for Odmadur 2, which did not fluctuate from the average in either direction to such a great extent as Odmadur 1.

The varieties Martondur 1 and Martondur 2 are the product of the Martonvásár breeding programme. They have excellent productivity (Table 1), yielding more than 10 t/ha under experimental conditions in 1994. Such high yields have never been achieved in Martonvásár with other durum wheat varieties. Over the average of six years they yielded at the same level as Yubileinaya 50. Between 1992 and 1997 the other durum varieties all yielded less than the control, the difference being 15.13% for GK Basa, 13.16% for Parus, 13.25% for GK Tiszadur, 5.8% for Odmadur 1 and 8.12% for Odmadur 2. This means that Martondur 1 and Martondur 2 yielded 7–20% more than the other durum wheat varieties over a six-year average, and performed as well as the *T. aestivum* control. The aim of improving productivity has thus been achieved. However, since the bread wheat varieties now under cultivation (Fatima 2, Martonvásári 23, Mv Optima, Mv Pálma) are 10–20% more productive than Yubileinaya 50, the yields achieved with the new durum wheats still appear more modest, which means that the difference in yield in favour of bread wheat still remains.

Data provided by the National Institute for Agricultural Quality Control confirm the findings of these experiments: the winter durum wheat varieties introduced or bred by the Martonvásár institute were some 7–14% more productive than the control winter durum wheat varieties (Table 2). Odmadur 1 and Odmadur 2 exhibited especially high yield potential in 1993, when their better winter hardiness was a great advantage.

Yields of spring durum wheat varieties

When the weather conditions in autumn are unfavourable for seedbed preparation and sowing there is often a need for spring wheat varieties, despite the fact that spring wheats rarely do well in Hungary. Some data from experiments set up with spring durum wheat varieties are presented in Table 3.

Table 2
Grain yields of winter durum wheat varieties
(Data from the National Institute for Agricultural Quality Control, 1993–1995)

Year	Odmadur 1		Odmadur 2		Martondur 1		Martondur 2		Mean of standard varieties	
	t/ha	%	t/ha	%	t/ha	%	t/ha	%	t/ha	%
1993	4.17	128.31	3.59	110.46					3.25	100.00
1994	7.17	114.72	6.97	111.44	7.34	117.44	6.06	96.96	6.25	100.00
1995	5.97	103.85	6.21	111.70	6.13	111.25	6.58	119.42	5.51	100.00
Mean										
1993–1995	5.77	115.16	5.59	111.57					5.01	100.00
1994–1995	6.57	111.73	6.59	112.07	6.74	114.54	6.32	107.48	5.88	100.00

The yields of the spring durum wheat varieties are compared to those of the winter wheat varieties Yubileinaya 50 (*T. aestivum*) and GK Basa (*T. durum*). Produra and Aldura had very poor productivity under Hungarian conditions, yielding 70% less than the control in 1983 and 1984, when the weather was unfavourable for spring wheats.

The yield data of two other spring durum wheat varieties are presented for 1989 and 1990. The more productive variety Po gave a yield equal to that of GK Basa over a two-year average, but this was still 27% less than the control.

Table 3
Grain yields of spring durum wheat varieties (Martonvásár, 1983–1997)

Variety	t/ha			%		
	1983	1984	Mean	1983	1984	Mean
Yubileinaya 50	7.58	7.01	7.29	100.00	100.00	100.00
GK Basa	6.01	7.18	6.59	79.29	102.43	90.39
Produra	2.58	1.42	2.00	34.03	20.39	27.43
Aldura	2.25	1.53	1.89	29.68	21.82	25.92
	1989	1990	Mean	1989	1990	Mean
Yubileinaya 50	5.81	6.05	5.93	100.00	100.00	100.00
GK Basa	4.94	3.82	4.38	85.03	63.14	73.86
Po	4.71	4.07	4.39	81.06	67.27	74.03
Mex. 75	4.28	3.77	4.02	73.66	62.31	67.87
	1996	1997	Mean	1996	1997	Mean
Yubileinaya 50	6.21	7.71	6.96	100.00	100.00	100.00
GK Basa	3.25	8.65	5.95	52.33	112.19	85.56
Topdur	4.72	4.63	4.67	76.01	60.05	67.09
Helidur	4.36	4.65	4.50	70.21	60.31	64.65
Astrodur	3.91	4.85	4.38	62.96	62.31	62.93
Semperdur	3.80	4.89	4.34	61.19	63.42	62.35
Marfil	3.58	4.78	4.18	57.64	61.99	60.05
Lona	4.51	5.30	4.90	72.62	68.74	70.40

Very similar yields were given by the spring durum wheat varieties tested over the last two years (1996 and 1997). As can be seen in Table 3, the most productive variety Topdur yielded 17.97% less than GK Basa and 32.91% less than Yubileinaya 50. In the majority of cases (6 years, 9 durum varieties) the spring durum yields were poorer than those of the autumn varieties.

The yield data of the spring bread wheat variety Lona (*T. aestivum*) are presented in the last row of Table 3. Although it gave a satisfactory yield in both years, it was still 29.60% behind Yubileinaya 50 and 15.16% behind GK Basa. Nevertheless, it yielded 3–10% better than the spring durum wheats.

On the basis of the data the following decreasing order was obtained for productivity: winter bread wheat, winter durum, spring bread wheat, spring durum.

The results confirmed earlier findings by the authors (Szunics et al., 1985) and others (Szabó, 1981; Beke and Matuz, 1982) to the effect that high-yielding common winter wheat varieties are more productive than winter durum wheats. The national durum wheat yield average of 6–8 t/ha that Szániel (1982) hoped to achieve has never become reality. According to Beke and Matuz (1996) the highest yield, amounting to 4.5 t/ha, was harvested in 1994. In experiments carried out by Grábner (1916) the spring durum varieties yielded more than common spring wheats, but this was not reflected in the present work, nor by that of Beke and Matuz (1996). The common spring wheat variety (Lona) gave a lower yield than the common winter wheat (Yubileinaya 50), thus confirming the well-known fact that under Hungarian conditions the winter forms of cereals are generally more productive than their spring counterparts.

Quality

A substantial proportion of the grain yield of durum wheat is used as raw material for pasta production. The breeding, introduction and cultivation of durum wheat is aimed at supplying the pasta industry with a satisfactory quantity and quality of home-grown raw material for the manufacture of pasta which will be readily marketable both in Hungary and abroad. Durum wheat marketed for milling and other food industry purposes must meet the requirements laid down in the state standard. If the quality of the yield does not come up to standard it can only be sold as fodder wheat.

It can be seen from the data in Table 4 that, when grown with the correct technology, the quality parameters of the registered varieties conform with the standard. However, all the quality characters exhibit great variation, ranging from very poor to excellent. This can be attributed to the fact that under Hungarian conditions good quality depends not only on the genetic potential of the variety, but also to a great extent on the growing site, production technology and climatic factors. These wide fluctuations may also be influenced by the fact that, according to Erdei and Gyenes (1981), Hungary represents the northern limit for the production of durum wheat. Quality may also be substantially reduced by spike fusarium, black point, lodging and delayed harvesting. Frequent rainfall when the wheat is ripe for harvesting will lead to a steep decline in quality, with reductions in vitreousness, hectolitre mass, semolina yield and yellow pigment content.

Table 4
Effect of climatic conditions on certain quality characters (Martonvásár, 1993–1997)

Character	GK Tiszadur	Odmadur 1	Odmadur 2	Martondur 1	Martondur 2
Vitreousness, %	12.0–97.0	19.0–94.0	16.0–92.0	24.0–95.0	38.0–100.0
Protein, %	11.5–13.4	11.9–14.8	11.1–13.8	12.7–15.1	12.5–15.5
Wet gluten, %	24.7–37.1	24.2–36.5	23.3–37.6	24.7–42.7	23.9–37.5
Dry gluten, %	8.2–13.2	10.1–13.0	8.2–12.8	8.8–14.6	8.4–12.8
Yellow pigment, mg/kg	5.3–7.5	6.5–7.3	6.1–6.5	5.7–8.1	6.3–8.3
Minolta „b" index	16.7–21.7	16.3–23.5	16.4–24.5	14.5–22.4	15.4–25.6

Frost resistance and winter hardiness

As the result of systematic work, breeders succeeded in developing winter durum wheats (Lukyanenko, 1967) which surpass the earlier spring types in both yield and yield stability. The Hungarian durum varieties are winter or alternate forms (Beke and Matuz, 1982) with much poorer winter hardiness than that of the bread wheats grown in Hungary (Szabó, 1981). In the 1978/79 season the severe winter led to considerable losses (Beke, 1980), which were repeated in the winter of 1992/93 (Szunics, 1996). Due to its poor winter hardiness Erdei and Gyenes (1981) and Beke and Matuz (1982) considered that it could only be grown reliably in the southern part of the country.

It is obvious from the above that the improvement of frost resistance and winter hardiness is a priority in breeding under Hungarian conditions. The phytotron facilities available in the institute make it possible to test and select genotypes for frost resistance every year, so that frost-sensitive forms can be eliminated. The frost resistance values recorded in the phytotron and in box experiments are presented in Table 5. The majority of the durum wheat varieties survived a temperature of –12°C at the tillering node in the absence of snow cover without any great damage, similarly to the bread wheat variety Bánkúti 1201. This means that in mild winters, when the fields are covered with snow, durum wheats give acceptable yields.

At a temperature of –13°C differences were found between the varieties. With the exception of Martondur 1 and Odmadur 2 the durum wheats were more sensitive to cold than Bánkúti 1201. At both freezing temperatures, however, the killed plant rate for durum wheats was lower than that of the winter barley Kompolti Korai. Despite the fact that durum wheats may suffer considerable damage in moderately severe winters, particularly if there is no snow cover, the cultivation of genotypes with frost resistance equivalent to that of moderately frost-resistant winter wheats or of the most frost-resistant winter barleys can be recommended under conditions suitable for the cultivation of winter barley provided they have satisfactory agronomic traits and there is a demand for their yield. They can also be used as basic breeding stock. Under more severe conditions (–14°C or below) they suffer substantial damage, especially if there is no snow cover. The data recorded in the box experiments on winter hardiness fell

Table 5
Frost sensitivity of winter durum wheat varieties (killed plant rate, %)
(Martonvásár, mean of three experiments)

Variety	Phytotron						Box
	−12°C	−12°C and −10°C	−13°C	−13°C and −10°C	−14°C	−15°C	
Triticum durum							
Odmadur 1	11.9	79.6	27.9	92.5	43.3	42.3	30.3
Odmadur 2	8.7	87.4	21.1	92.2	53.2	41.1	16.7
Martondur 1	7.6	70.9	18.5	85.0	43.8	53.8	9.6
Martondur 2	22.5	89.8	55.4	89.6	93.8	95.0	37.2
GK Tiszadur	15.4	98.7	36.3	90.0	68.4	62.8	18.1
Triticum aestivum							
Bánkúti 1201	10.1	55.1	20.9	79.3	51.8	47.5	14.1
Hordeum vulgare							
Kompolti korai	46.3		89.7	100.0	98.7	98.6	

between those measured at −12°C and −13°C in the phytotron and gave the same order for the varieties with the exception of Odmadur 1.

After the end of the vernalisation period wheat loses its frost resistance. The vernalisation requirement of durum wheats is shorter than that of cultivated bread wheat varieties. It often happens in Hungary that the weather warms up in the second half of February, leading to the initiation of vegetation. This is then followed in early March by the return of colder weather, at which time many wheat crops suffer damage. According to Veneny (1939) it is not so much the winter frosts that damage durum varieties as the late frosts in spring. An experiment was thus set up to investigate the effect of such late frosts on the plants, by refreezing them at −10°C after initial freezing at −12°C or −13°C. Such conditions were extremely unfavourable for durum wheat and winter barley. Even the common wheat Bánkúti 1201 suffered considerable damage. In general, the genotypes examined suffered greater frost kill after refreezing than after freezing at −14°C or −15°C. The genetic frost resistance of the varieties may be manifested in nature in various ways. The effect of severe winters and that of repeated freezing in early spring may both be considerable.

Disease resistance

Yield stability depends to a great extent on the resistance of the varieties to diseases. One safe, successful, economical and environmentally sound means of protection is to breed and grow resistant varieties.

Durum wheat may be susceptible to numerous fungal, bacterial and viral diseases throughout its development, from germination to full maturity. Observations over the last 15 years indicate that the majority of durum wheat

varieties are infected by all the pathogens that affect common wheat. For this reason, mention will only be made of diseases which require more than average attention during the breeding and cultivation of durum wheat.

In the present experiments most durum wheat varieties proved to be very sensitive to **spike fusarium**. While Yubileinaya 50 exhibited an infection rate of 3.3% over a three-year average, Odmadur 2 and Odmadur 1 had infection rates of 13.3% and 33.1%, respectively. The latter variety suffered 49.7% infection in 1990. Other even more susceptible varieties were also found, such as Kristall in 1985 (59.5%) and Korall in 1987 (97.2%). Mesterházy (1995) also emphasised the greater susceptibility of durum wheats. As there are no varieties resistant to this pathogen and no effective means of chemical control (Kükedi, 1977; Fabriani and Lintas, 1988), substantial damage is likely (yield losses, quality deterioration, mycotoxin production) in the case of epidemics. The most frequent pathogens are *Fusarium graminearum, F. culmorum* and *F. avenaceum* (Békesi and Hinfner, 1971; Szunics et al., 1978; Mesterházy, 1984), while Tóth (1991) detected the multiplication of toxin-producing species such as *F. sporotrichioides, F. poae* and *F. semitectum*.

Snow mould, caused principally by *F. nivale*, is not a frequent pathogen in Hungary. In 1996 and 1997, however, the weather was favourable for the pathogen and considerable infection was observed in winter cereals (Szunics et al., 1997). The investigations showed that only 3 of 36 winter durum wheats (8.3%) remained symptom-free. Twelve genotypes (33.5%) were severely infected. It should be noted that Veneny remarked on the greater sensitivity of durum wheats to snow mould as long ago as 1939.

Black point can be caused not only by genetic and physiological effects, but also by various fungi. Species belonging to the *Alternaria* genus, especially *A. alternata*, are isolated most frequently from wheat grains exhibiting symptoms of the disease. In addition, many saprophytic fungi belonging to the *Cladosporium, Aspergillus, Gonatobotrys* and *Epicoccum* genera and a few potentially pathogenic species (*Bipolaris sorokiniana, Drechslera biseptata, Fusarium graminearum, F. moniliforme*) have been identified. Black point causes a serious reduction in the pasta-making quality of durum wheat. It is thus an important selection trait in the Martonvásár breeding programme, especially in the light of the fact that susceptibility is inherited (Fabriani and Lintas, 1988). Considerable differences were observed between the genotypes; in 1992, for instance, GK Tiszadur was symptom-free, while the rate of infection was 6.2% for Odmadur 1, 21.5% for Chernomor and as high as 35.0% for TDB 492–92 (Fischl et al., 1993).

On occasion **viruses** may also cause substantial damage. A relatively high degree of virus infection was observed in the institute's nurseries in 1972, 1978, 1982, 1983, 1986, 1990 and 1996. Under Hungarian conditions the **barley yellow dwarf** Luteovirus (BYDV), first identified in 1966, used to be the most widespread and the most familiar. The 1996 epidemic, however, was caused by

the intense multiplication of the **wheat dwarf** Gemini virus (WDV), isolated and identified in Hungary in 1988. Among the plants showing symptoms of virus infection, 70.5% were found to be infected with WDV and 12.0% with BYDV (Pocsai et al., 1997).

In 1996, chiefly as the result of the WDV infection, the winter durum wheat experiments suffered substantial damage in one nursery and remained free of symptoms in the other. By means of a series of complex calculations this made it possible to estimate the damage. The extent of virus infection was recorded on two occasions (May 23rd and June 12th 1996). By the end of the vegetation period the extent of virus infection had increased. The different genotypes responded differently to the pathogen attack. None of the varieties were really resistant, though Odmadur 2 and Odmadur 1 had scores of 1.8 and 2.2, respectively, compared to the 1.5 score recorded for Yubileinaya 50. Martondur 1 (2.5) and Parus (3.5) were somewhat more sensitive, while Martondur 2 (8.5) and GK Basa (8.7) were very susceptible.

For genotypes which were symptom-free at the first scoring date but exhibited a slight degree of infection on the second occasion, an average yield loss of 3.1% was recorded (Table 6). The yield losses increased with the degree of susceptibility, but even lines with the same scores did not respond uniformly to the pathogen. In the 4.1–6.0 scoring category, for instance, the yield loss averaged 22.2%, with values ranging from 12.5% for lines less sensitive to infection to 45.7% for the most sensitive lines. Genotypes given a score of 5 or more at the first scoring date had generally become completely infected by the second scoring date, which meant that most of the plant stand, and thus most of the yield was destroyed. In some plots 100–200 g of worthless seed was harvested in place of the usual 5–6 kg. Plots with scores of 8 or 9 gave virtually no yield.

Table 6
Possible yield losses (%) due to virus infection (Martonvásár, 1996)

Degree of infection	No. of samples	Possible yield losses (%)	
		Mean	Interval
0.0–2.0	18	3.1	00.0–07.5
2.1–4.0	25	7.3	00.0–12.9
4.1–6.0	7	22.2	12.5–45.7
6.1–7.0	5	39.4	26.7–64.9
7.1–8.0	11	60.5	47.8–81.9
8.1–9.0	6	84.6	58.4–97.0

What are the advantages of growing the new varieties?

The development of biologically more valuable genotypes represents *scientific* progress, since they combine better productivity and winter hardiness and, since they can be grown more reliably due to their better adaptability, are suitable for cultivation on larger areas.

The competitiveness of up-to-date genotypes leads to **economic** advantages, both **directly** (higher yields, larger sowing area, better markets for seed and grain, semolina yield of good quality for pasta-making, marketability, price difference, saving on the price of the eggs eliminated from the pasta-making technology, replacement of imports, possibility of exporting) and **indirectly** (human-orientated, hygienic, better storability).

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PRODUCTION AND PROCESSING VALUES OF DIFFERENT GENOTYPES OF WHEAT GROWN IN YUGOSLAVIA

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The grain yield of 19 wheat varieties grown at 18 localities in Yugoslavia was studied over a 3-year period. The winter wheat varieties investigated are higher yielding than the standards (Yugoslavia, Partizanka, Super Zlatna, Zlatna Dolina) by amounts ranging from 17 kg/ha (PKB-Talas) to 1,251 kg/ha (BG-Maksima). It should be added, however, that the variety PKB-Talas is of blending quality, BG-Maksima is of breadmaking quality, while Super Zlatna is used for feeding.

In relation to the quality indexes (% crude protein, sedimentation values and bread yield) the varieties PKB-Krupna and PKB-111 are on the level of standards having high yields but poor quality (Zlatna Dolina and Super Zlatna), PKB-40, PKB-Lepoklasa, PKB-Vizeljka, PKB-Crvena, BG-Carica, BG-Merkur, BG-Maksima, PKB-Sunce, PKB-Prelivka, PKB-Lepostasa and BG-Vitka are on the level of a standard with medium quality (Yugoslavia), while the varieties PKB-Padinka, PKB-Talas, PKB-Rana, PKB-Ratarica and PKB-Mlinarka are on the level of a standard with excellent quality (Partizanka).

Key words: wheat, variety, grain yield, grain quality

Introduction

Bearing in mind that there is less than 0.4 ha of arable land per person in the world and that the reserves of potentially arable land are double this at the most, one solution for feeding the rapidly increasing population of the world in the near future is the permanent increasing of the cultured plants per unit area, of which wheat is very important because many products necessary for human nutrition are made from wheat grain.

An increase in wheat production depends, first of all, on the creation of new varieties with high genetic potential for yield and grain quality, and also on the development and application of technologies that will facilitate the maximum use of the given genetic potential. In this century, especially over the last few decades, outstanding results have been reached in this direction. With new wheat varieties in large-scale production, yields of 8.0, 9.0 or even 10.0 t/ha are obtained.

All the success achieved in wheat production is the result of maniy factors, of which genetic factors and environmental factors are crucial.

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Materials and methods

The trials were set up in a block design with five replications. The basic plot size was 5 m^2. Basic tillage was carried out on the "till, prepare and sow" system. The number of localities was eighteen. The soil type differed from locality to locality. Sowing was carried out by hand or mechanically, with a row spacing of 20 cm, 600 germinating grains per m^2, and the usual agricultural techniques employed for winter wheat in Yugoslavia. Sowing time was the middle of October.

The experimental material consisted of winter wheat varieties developed in the "PKB-INI Agroekonomik" Institute (PKB-Krupna, PKB-40, PKB-111, PKB-Padinka, PKB-Lepozrna, PKB-Lepoklasa, PKB-Vizeljka, PKB-Crvena, PKB-Vitka, PKB-Prelivka, PKB-Rana, PKB-Ratarica and PKB-Lepostasa), sown in three vegetation periods (1993/94–1995/96). The varieties Partizanka, Yugoslavia, Super Zlatna and Zlatna Dolina were used as standards.

On the basis of protein content and sedimentation value Partizanka belongs to quality class I. It has excellent milling and baking properties. The variety is productive, with a genetic grain yield potential of over 8.0 t/ha. It is a very plastic variety. It is grown in most of the cereal-growing areas of Yugoslavia. Since 1976 this variety has been the standard for varieties of very good technological quality in trials set up in Yugoslavia.

On the basis of protein content and sedimentation value the variety Yugoslavia belongs, mostly, to quality class II. It has very good milling and baking quality. It is an excellent bread wheat and is very productive, with a genetic grain yield potential of over 10.0 t/ha. It is a fairly plastic variety. It is becoming popular in all the wheat-growing areas of Yugoslavia, where it occupies significant areas. Since 1988 it has served as a standard in Yugoslavian trials.

On the basis of protein content and sedimentation value the variety Super Zlatna belongs to quality class III. It has unsatisfactory technological properties. It is a very productive variety with a genetic grain yield potential of over 10.0 t/ha. It can be found in production on intensive areas of Yugoslavia.

The protein content and sedimentation value of the variety Zlatna Dolina place it in quality class III. It has unsatisfactory milling and baking properties. It is a very productive variety with a genetic grain yield potential of over 9.0 t/ha.

The crude protein content was determined according to the Yugoslav standard method JUS E.B8.031 (macro-Kjeldahl) dated 1978, using an automatic KJELTEC device manufactured in Sweden. The results are expressed as a percentage of the dry matter content.

The sedimentation value of the wheat varieties was determined by the Yugoslav standard method JUS E.B8.030 dated 1978. The wheat grains were crushed and sieved through a special mill, and the wheat flour obtained was suspended in lactic acid solution. The sedimentation values were read after a certain time and the results were expressed as percentages.

The bread volume was established twenty-four hours after baking as the product of the average bread mass and the dough yield, divided by the mass of dough. The results were expressed as g of bread per 100 g of wheat flour.

The results are shown as a three-year average.

Results and discussion

Grain yield

In wheat production, in addition to the ecological conditions and the level of technology applied, great importance is also attached to varieties with high potential for grain quantity and quality, adapted to the different agroecological conditions of the country. The creation of varieties with high genetic grain yield

potential and very good milling and baking quality is one of the main breeding tasks (Borojević, 1978; Mišić and Mikić, 1976).

The results of the investigations showed that the varieties tested had grain yields higher than those of the standard by amounts ranging from 17 kg/ha (PKB-Talas) to 1,251 kg/ha (BG-Maksima). It should be noted that the variety PKB-Talas is classified as a blending variety (Class I) and BG-Maksima as a bread wheat, while the varieties Zlatna Dolina and Super Zlatna (Class III) are classified according to Yugoslavian standards as "basic" (Table 1).

Table 1

Grain yield (kg/ha) of different winter wheat genotypes (averaged over 3 years and 16 locations)

Variety	av. for 3 years	DP*	DY*	DZ*	DSZ*
PKB-Krupna	6,523	$+353^{++}$	–	$+195^{+}$	–
PKB-40	6,427	$+360^{++}$	–	$+402^{++}$	–
PKB-111	6,021	$+110^{+}$	–	$+427^{++}$	–
PKB-Padinka	6,463	$+152^{+}$	–	$+452^{++}$	–
PKB-Lepozrna	6,350	$+156^{++}$	–	$+371^{++}$	–
PKB-Lepoklasa	6,538	$+171^{++}$	–	$+485^{++}$	–
PKB-Vizeljka	6,414	$+179^{++}$	–	$+372^{++}$	–
PKB-Crvena	6,412	$+499^{++}$	–	–	$+444^{++}$
PKB-Talas	6,261	$+279^{++}$	–	–	$+17^{ns}$
PKB-Mlinarka	6,542	$+733^{++}$	–	–	$+249^{++}$
BG-Carica	7,204	$+788^{++}$	$+169^{++}$	$+442^{++}$	–
BG-Merkur	7,111	$+661^{++}$	$+57^{ns}$	$+447^{++}$	–
BG-Maksima	7,667	$+1251^{++}$	$+629^{++}$	$+922^{++}$	–
PKB-Sunce	7,745	$+760^{++}$	$+249^{++}$	–	–
BG-Vitka	7,773	$+814^{++}$	$+477^{++}$	–	–
PKB-Prelivka	7,723	$+813^{++}$	$+457^{++}$	–	–
PKB-Rana	7,514	$+491^{++}$	$+347^{++}$	–	–
PKB-Ratarica	7,328	$+395^{++}$	$+184^{++}$	–	–
PKB-Lepostasa	7,595	$+572^{++}$	$+428^{++}$	–	–

DP* = Difference between investigated variety and blending variety Partizanka
DY* = Difference between investigated variety and wheat variety Yugoslavia
DZ* = Difference between investigated variety and wheat variety Zlatna Dolina (technological group III)
DSZ* = Difference between investigated variety and wheat variety Super Zlatna (technological group III)
+ = Difference significant at P = 0.05
++ = Difference significant at P = 0.01
ns = Not significant.

The grain yield of the variety PKB-Mlinarka was 733 kg/ha higher than that of Partizanka, while that of PKB-Talas was 279 kg/ha higher, but both varieties are in the same quality class (Tables 1 and 2). The variety BG-Maksima achieved an average yield of 7,667 kg/ha. This variety yielded 1,251 kg/ha more than Partizanka, 629 kg/ha more than Yugoslavia and 922 kg/ha

more than Zlatna Dolina. BG-Carica achieved an average yield of 7,204 kg/ha, i.e. 788 kg/ha more than the yield of Partizanka, 169 kg/ha more than Yugoslavia and 422 kg/ha more than Zlatna Dolina. BG-Merkur achieved a yield of 7,111 kg/ha. This is 660 kg/ha more than the yield of Partizanka, 57 kg/ha more than the yield of Yugoslavia and 477 kg/ha more than the standard Zlatna Dolina. PKB-Lepoklasa, with an average yield of 6,538 kg/ha, yielded 485 kg/ha more than Zlatna Dolina and 171 kg/ha more than the standard variety Partizanka. The variety PKB-Sunce had an average grain yield of 7,745 kg/ha, 760 kg/ha higher than that of Yugoslavia (Table 1).

The grain yield of the newly created variety PKB-Prelivka averaged over five locations and three years of investigation, was 7,723 kg/ha, i.e. 457 kg/ha higher than that of Yugoslavia and 813 kg/ha higher than that of Partizanka (Table 1).

The variety PKB-Rana had an avarage grain yield of 7,514 kg/ha, 347 kg/ha higher than Yugoslavia and 491 kg/ha higher than Partizanka (Table 1).

The new variety PKB-Ratarica had an average grain yield of 7,328 kg/ha, 184 kg/ha higher than Yugolavia and 395 kg/ha higher than Partizanka (Table 1).

PKB-Lepoklasa achieved an average grain yield of 7,595 kg/ha, 428 kg/ha higher than Yugoslavia and 572 kg/ha higher than Partizanka (Table 1).

Approximately the same relationship was observed between the varieties and standards investigated in relation to grain yield in investigations at one location in micro- and macro-trials by several authors (Protić, 1990; 1993; Protić and Pavlović, 1994).

Crude protein

The crude protein content of the investigated varieties was the lowest in BG-Vitka (11.5%) and the highest in the varieties PKB-Crvena (14.5%), BG-Merkur (14.3%) and BG-Carica (14.3%). High crude protein contents were recorded for PKB-Talas (14.1%), PKB-Padinka (13.8%), PKB-40 (13.4%), PKB-111 (13.5%) and BG-Maksima (13.4%) (Table 2).

In relation to the standards (Partizanka, Yugoslavia, Zlatna Dolina and Super Zlatna) the results show that, on the basis of crude protein content, the varieties PKB-Krupna and PKB-111 are similar to Zlatna Dolina and Super Zlatna and, like them, belong to the basic varieties or quality class III. The varieties PKB-40, PKB-Crvena, BG-Carica, BG-Merkur, BG-Maksima, PKB-Sunce, PKB-Prelivka, PKB-Lepostasa and BG-Vitka have crude protein contents similar that of Yugoslavia, and are thus bread wheat varieties belonging to quality class II. PKB-Padinka, PKB-Lepozrna, PKB-Talas, PKB-Rana, PKB-Ratarica and PKB-Mlinarka have crude protein contents similar to that of Partizanka and are thus blending varieties belonging to quality class I. PKB-Lepoklasa and PKB-Vizeljka have somewhat better crude protein contents than Yugoslavia, but are poorer than Partizanka and can thus be classified as bread varieties in quality class II (Table 2).

Table 2
Crude protein content (% of dry matter) of different winter wheat varieties (averaged over 3 years and 5 locations)

Variety	Investigated variety	Partizanka	Yugoslavia	Zlatna Dolina	Super Zlatna	$LSD_{0.05}$	$LSD_{0.01}$
PKB-Krupna	12.8	13.3	–	11.7	–	0.85	1.11
PKB-40	13.4	13.9	–	11.8	–	0.75	1.12
PKB-111	13.5	14.0	–	11.9	–	0.65	0.90
PKB-Padinka	13.8	12.8	–	11.8	–	0.61	0.80
PKB-Lepozrna	13.5	12.8	–	11.8	–	0.61	0.80
PKB-Lepoklasa	12.6	13.0	–	11.8	–	1.03	1.36
PKB-Vizeljka	12.6	13.0	–	11.8	–	1.03	1.36
PKB-Crvena	14.4	14.7	–	–	14.1	0.72	0.96
PKB-Talas	14.1	14.0	–	–	13.1	0.88	1.16
PKB-Mlinarka	13.8	13.9	–	–	13.1	0.95	1.25
BG-Carica	14.3	14.2	13.4	13.0	–	1.40	1.70
BG-Merkur	14.3	14.2	13.4	13.0	–	1.40	1.70
BG-Maksima	13.4	14.8	13.6	12.6	–	1.47	2.49
PKB-Sunce	12.2	14.8	12.7	–	–	0.61	0.83
BG-Vitka	11.5	12.9	12.8	–	–	0.60	0.84
PKB-Prelivka	12.0	13.4	13.2	–	–	1.16	1.58
PKB-Rana	13.8	13.4	13.2	–	–	1.16	1.58
PKB-Ratarica	13.2	13.4	13.2	–	–	1.16	1.58
PKB-Lepostasa	12.6	13.4	13.2	–	–	1.16	1.58

Sedimentation value

The sedimentation values of the varieties investigated ranged from 30 (PKB-111) to 56 (PKB-Rana). In relation to the standards (Partizanka, Yugoslavia, Zlatna Dolina, Super Zlatna) the results show that varieties PKB-Krupna, PKB-111, PKB-Crvena and BG-Carica have sedimentation values at the level of Zlatna Dolina and Super Zlatna. Varieties PKB-Padinka, PKB-Lepozrna, PKB-Lepoklasa, PKB-Vizeljka, PKB-Talas, PKB-Mlinarka, PKB-Rana and PKB-Ratarica have values similar to that of Partizanka, while BG-Merkur, BG-Maksima, PKB-Sunce, PKB-40, BG-Vitka, PKB-Padinka and PKB-Lepostasa resemble the bread variety Yugoslavia (Table 3). Similar results were reported by Šarić (1994) after investigations on the varieties PKB-Padinka, PKB-Lepoklasa, PKB-Sunce, PKB-Krupna, PKB-111 and PKB-Mlinarka.

Bread yield

The bread yield of the varieties investigated ranged from 134.9 (BG-Vitka) to 141.5 g/100 g of flour (PKB-Padinka). Good bread yields were also recorded for PKB-Lepoklasa (141.2 g/100 g of flour) and for PKB-Mlinarka, PKB-Talas and PKB -Sunce (Table 4). With the exception of the varieties PKB- Krupna,

Table 3
Sedimentation value and quality class of different winter wheat genotypes (averaged over 3 years and 5 locations)

Variety	Sedimentation value					Quality class*
	Investigated variety	Partizanka	Yugoslavia	Zlatna Dolina	Super Zlatna	
PKB-Krupna	32	52	–	25	–	III
PKB-40	38	49	–	28	–	II
PKB-111	30	50	–	29	–	III
PKB-Padinka	50	48	–	25	–	I
PKB-Lepozrna	48	48	–	23	–	I
PKB-Lepoklasa	55	50	–	22	–	II
PKB-Vizeljka	50	50	–	22	–	II
PKB-Crvena	26	57	–	–	–	II
PKB-Talas	47	53	–	–	27	I
PKB-Mlinarka	49	54	–	–	25	I
BG-Carica	32	48	32	27	28	II
BG-Merkur	39	49	32	27	–	II
BG-Maksima	36	54	36	28	–	II
PKB-Sunce	41	61	41	–	–	II
BG-Vitka	41	46	36	–	–	II
PKB-Prelivka	43	59	42	–	–	II
PKB-Rana	56	59	42	–	–	I
PKB-Ratarica	47	59	42	–	–	I
PKB-Lepostasa	43	59	42	–	–	II

* According to Yugoslavian recommendations

PKB-111 and BG-Vitka, which had bread yields similar to those of Zlatna Dolina and Super Zlatna, the other varieties investigated gave results similar to that of the standard Partizanka (Table 4). When investigating the varieties PKB-Padinka, PKB-Lepoklasa, PKB-Sunce and PKB-Mlinarka, Šarić (1994) found similar bread yields.

Conclusions

The grain yields of the wheat varieties investigated were higher than those of the standards by amounts ranging from 17 kg/ha (PKB-Talas) to 1,251 kg/ha (BG-Maksima).

On the basis of crude protein content, the varieties PKB-Krupna and PKB-111 resembled Zlatna Dolina and Super Zlatna (Class III). The varieties PKB-40, PKB-Crvena, BG-Carica, BG-Merkur, BG-Maksima, PKB-Sunce, PKB-Prelivka, PKB-Lepostasa and BG-Vitka had values similar to that of the variety Yugoslavia. PKB-Padinka, PKB-Lepozrna, PKB-Rana, PKB-Talas and PKB-Mlinarka had crude protein contents resembling that of Partizanka, while the values of PKB-Lepoklasa and PKB-Vizeljka fell between those of Partizanka and Yugoslavia.

Table 4
Bread yield (g/100 g flour) of different winter wheat genotypes (averaged over 3 years and 5 locations)

Variety	Investigated variety	Partizanka	Yugoslavia	Zlatna Dolina	Super Zlatna	$LSD_{0.05}$	$LSD_{0.01}$
PKB-Krupna	136.7	139.4	–	137.1	–	2.01	2.64
PKB-40	140.0	139.2	–	136.2	–	1.89	2.35
PKB-111	136.1	139.0	–	137.0	–	1.34	2.01
PKB-Padinka	141.5	139.8	–	136.3	–	1.18	1.58
PKB-Lepozrna	140.6	139.8	–	136.3	–	1.19	1.58
PKB-Lepoklasa	141.2	139.8	–	136.1	–	1.13	1.49
PKB-Vizeljka	140.6	139.8	–	136.1	–	1.13	1.49
PKB-Crvena	136.8	138.7	–	–	135.0	1.39	1.83
PKB-Talas	135.8	135.1	–	–	133.2	1.52	2.01
PKB-Mlinarka	137.2	136.3	–	–	135.0	1.11	1.47
BG-Carica	136.6	137.0	136.3	135.6	–	3.93	4.79
BG-Merkur	138.5	137.0	136.3	135.6	–	3.93	4.79
BG-Maksima	130.0	137.7	135.8	135.6	–	3.00	5.10
PKB-Sunce	138.5	138.7	136.5	–	–	2.11	2.88
BG-Vitka	134.9	138.7	134.6	–	–	2.73	3.83
PKB-Prelivka	138.1	137.9	137.9	–	–	2.26	3.09
PKB-Rana	.139.8	137.9	137.9	–	–	2.26	3.09
PKB-Ratarica	138.1	137.9	137.9	–	–	2.26	3.09
PKB-Lepostasa	140.8	137.9	137.9	–	–	2.26	3.09

The sedimentation values of the varieties investigated ranged from 30 (PKB-111) to 56 (PKB-Rana), and their bread yields from 134.9 (PKB-Vitka) to 141.5 g/100 g of flour (PKB-Padinka).

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CROP NUTRITIONAL STATUS OF MAIZE AND SOYBEAN IN FLORIDA

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The nutrient sufficiency status of maize (*Zea mays* L.) and soybean (*Glycine max* L. Merr.) on Arredondo sandy soil (loamy siliceous, hyperthermic, Arenic and Grossarenic Paleudult) as affected by seven different cropping histories and by the cultivar of soybean were studied at the Green Acres Agronomy Farm, University of Florida, in 1991. Parallel analyses were carried out on leaf tissue and soil samples to determine the concentrations of N, P, K, Ca, Mg, Cu, Fe, Mn and Zn, and the pH and organic matter content of the soil. N was deficient in maize but sufficient in soybean at all locations. Ca, P and Fe were sufficient for both crops at all locations. K, Mg, Mn, Zn and Cu were either low, sufficient or high depending on the crop, cultivar or crop rotation. The two soybean cultivars differed in the leaf concentrations of P at four different crop rotation sites and of Cu at only one location. Significant positive correlations ($P < 0.01$) existed between the soil element content and the corresponding concentration of K, P and Mg in maize and Zn and Cu in soybean. A review of current fertiliser application frequency and amount on Arredondo loamy sand and the periodic use of diagnostic plant analysis on similar sandy soils are recommended.

Key words: nutrient sufficiency, maize, soybean, Arredondo sandy soil, crop rotation

Introduction

Crop nutrition is important for the yield of crops and the ultimate returns from agricultural crop production. The monitoring role of plant analysis or a series of plant analyses is little used and understood; and yet it offers farmers the opportunity to maintain high quality production with a minimum of nutrient deficiency problems. Repeated plant analyses during the growth cycle of a plant or from one season to another can profile changes which are occurring with time as a result of fertilizer treatment. The need for supplemental fertilizer treatments can be determined in order to maintain a high level of productivity over a long growing season.

While the amount of fertilizer applied may not be an indicator of the nutrient concentration in the plant, the status of an element in the soil may relate to the nutritional status of the plant (Jones, 1974). Several difficulties have been encountered in the use and interpretation of plant analyses, even though the quantitative association between an absorbed essential element and plant growth has been widely studied (Jones et al., 1991). Initially, single concentration values, such as critical or standard concentrations were, sought, but current interpreters prefer to use data which list the full range of concentration from

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deficiency to excess. A critical value is the concentration below which deficiency occurs and is difficult to use if the assay concentration is considerably above or below the critical values. A different and fairly new concept of plant analysis interpretation is the Diagnosis and Recommendation Integrated System (DRIS) which is based on the principle of elemental interrelationships by determining, in ascending order, the most to the least limiting elements (Beaufils, 1973). Studies of established plant analysis programmes for maize and other crops, however, showed that a DRIS-based interpretation was no better than one based on sufficiency range techniques (Kelling and Schulte, 1986). Few laboratories are known to be using the DRIS technique (Savoy and Robinson, 1990).

None of the above interpretative procedures is infallible, and it has therefore been recommended that the interpreter of plant analysis must use all the resources available, including a soil test result, to determine the nutrient status of the plant in order to advise on treatments to correct detected insufficiencies (Jones et al., 1991). In diagnostic plant analysis, differences in the plant part, stage of growth, genotype and geographic location may cause variation in elemental concentrations in the plant.

The sufficiency status of N, P, K, Ca, Fe, Mn and Zn in leaves of maize (*Zea mays* L.) and soybean (*Glycine max* L. Merr.) was therefore determined as affected by location and the variety of soybean. The sufficiency status of N, P, K, Ca, Fe, Mn and Zn in the leaves of these crops was also evaluated in relation to Mehlich I extractable N, P, K, Ca, Fe, Mn and Zn, soil organic matter and pH to provide recommended measures to improve plant health if needed.

Materials and methods

Maize (Pioneer 3089) and soybean (Deltapine 105 and Howard) crops were planted at seven locations on the Green Acres Agronomy Farm, Institute of Food and Agriculture Science, University of Florida, in 1991. The experimental fields, located at Gainesville, were on an Arredondo sandy soil (loamy siliceous, hyperthermic, Arenic and Grossarenic Paleudult) (Soil Survey Staff, 1984). Each of the sites had different multiple cropping histories and the two crops immediately preceding these plantings are presented in the results. After conventional tillage of the sites, a Brown Harden in-row subsoil no-tillage planter was used to lay off rows. All crops were then planted by hand in a randomized complete block design at each location. Each plot consisted of four rows 0.75 m wide and 3.75 m long. Treatments were replicated four times. For crop nutrition evaluation each location represented a treatment for each individual crop analysis. These evaluations were treated as a randomized complete block for statistical purposes. For soybean the seven locations were treated as whole plots and the two cultivars were treated as split plots. The seeding rate for maize was $7/m^2$ and for soybean $40/m^2$. Eight ear leaves of maize at early silking (61 DAP) and 25 youngest mature leaves of soybean at early head emergence (58 DAP) were sampled. Soil samples were taken from the top 0.15 m at the same time as leaf sampling and processed for nutrient status. Fertilizer application followed the current recommended rates and sequence for use on Green Acres experimental fields for maize and soybean. The total fertilizer plant nutrients applied to the crops were N 185 kg/ha, P 5 kg/ha, K 100 kg/ha , S 55 kg/ha and Mg 54 kg/ha for maize, and N 27 kg/ha, P 5 kg/ha, K 100 kg/ha, S 55 kg/ha and Mg 54 kg/ha for soybean. Nitrogen was applied in four increments for maize and starter N was applied at planting for soybean. Greater pesticide management was required for maize than for soybean. Pesticides

were applied according to labelled rates, times and procedures. No herbicides were used in the study. Weeds were controlled by two hand weedings and two mechanical cultivations.

The plant leaves were dried in a forced air oven at 70°C for 24 hours until completely dry. The samples were then ground in a Wiley mill to pass a 2 mm stainless steel screen and stored in air-tight sterile plastic bags. All samples were opened and redried for four hours after being ground, and resealed immediately after removal from the oven. Plant samples weighing 100 mg were used for N determination according to procedures described by Gallahar et al. (1975). The samples were dry ashed and extracted into 0.1 N HCl (AOAC, 1990). The solutions were analysed for P by colorimetry, for K by flame emission and for Ca, Mg, Cu, Fe, Mn and Zn by atomic absorption spectrophotometry. The soil samples were allowed to air dry over a period of several days. A stainless steel screen with 2 mm size holes was used to screen the soil. This was followed by hand mixing the soil to ensure a representative sample during analysis. The samples were organized and stored in wooden plates until analysis was complete. The laboratory procedure for N analysis was identical to plant analysis except that 2.0 g of soil sample was used without glass beads. The soil samples were extracted by the acid procedure (Mehlich, 1954) and analysed for P, K, Ca, Fe, Mn and Zn as described for plant mineral analysis. Soil pH was determined with a 1:2 soil solution ratio in H_20 using a glass electrode. Soil organic matter (OM) was determined by a modified version of the Wakley-Black method (Allison, 1965).

Plant and soil analysis data were organized in spreadsheets and the appropriate analysis of variance and correlations conducted using MSTAT (1985). Duncan's test was applied to test mean differences. Crop nutrient interpretative values were compiled for each crop (Jones et al., 1991).

Results and discussion

The nutritional status of crops is dependent upon several factors, including crop species, genotype within a species, type of soil, cropping history, age of tissue sampled and amounts of fertilizer applied. In this study the nutritional relationships of maize and soybean were investigated on loamy sand with different cropping histories.

Table 1 shows the concentration of nutrients in the ear leaf of maize and the youngest mature leaves of soybean grown at seven locations. The ranges in nutrient levels for maize were as follows: Ca, 2.6–4.3 g/kg; Mg, 1.31–2.17 g/kg; K, 15.9–20.9 g/kg; P, 2.70–3.13 g/kg; N, 19.7–23.8 g/kg; Cu, 3.5–7.8 mg/kg; Fe, 62.5–80.0 mg/kg; Mn, 21.0–55.8 mg/kg; Zn, 17.0–29.5. The ranges in nutrient levels for the two soybean varieties were Ca, 6.30–7.60 g/kg; Mg, 3.51–3.99 g/kg; K, 5.1–17.8 g/kg; P, 2.91–3.40 g/kg; N, 43.4–48.2 g/kg; Cu, 3.15–7.25 mg/kg; Fe, 90.0–113.8 g/kg; Mn, 56.4–130.0 mg/kg; Zn, 33.8–72.8 mg/kg.

Based upon sufficiency values, the maize hybrid 'Pioneer 3098' used in the study had low levels of N in the ear leaf at all seven locations. Even though N fertilizer was applied at the normal rate with split applications, as currently recommended, the analysis revealed an insufficient level in maize leaves. Other nutrients that were low in the ear leaf included K at two locations, Mg at four of the seven locations, Cu at four locations and Zn at one location. All other nutrients were sufficient in maize ear leaves. Soybean leaves had sufficient to high levels of all the nutrients measured except for K and Cu. Sufficient K was only found in the leaves in the fallow/peanut and lupin/ vegetable crop rotation systems.

Table 1
Concentration of nutrients in the ear leaves of maize (Pioneer 3089) and youngest mature leaves of soybean (Deltapine and Howard) after different previous crops

Nutrient	Previous crops						
	Fallow/ Peanut	Wheat/ Maize	Clover/ Maize	Lupin/ Vegetables	Rye/ Maize	Vetch/ Maize	Lupin/ Maize
				Maize			
Ca (g/kg)	2.6cS	3.0bcS	4.3aS	3.4bcS	3.4bcS	3.9abS	3.4abcS
Mg (g/kg)	1.31cL	1.99bcS	2.73aS	1.85bcL	2.17abS	1.94bcL	1.66bcL
K (g/kg)	20.9aS	16.7bcL	15.9cL	20.1aS	17.4bcS	19.1abS	18.5abS
P (g/kg)	2.87aS	2.74aS	2.70aS	2.65aS	2.91aS	3.13aS	2.94aS
N (g/kg)	23.8aL	19.7bL	22.1abL	22.7abL	20.2bL	23.8aL	21.9abL
Cu (mg/kg)	4.5cL	3.8cL	3.5cL	7.8aS	4.5cL	7.0abS	6.3bS
Fe (mg/kg)	75.0aS	67.5aS	67.5aS	80.0aS	80.0aS	62.5aS	70.0aS
Mn (mg/kg)	34.0bS	30.5bS	21.0bS	27.3bS	26.5bS	48.3aS	55.8aS
Zn (mg/kg)	17.0bL	27.0aS	26.0aS	28.0aS	28.3aS	295.0aS	27.0aS
				Soybean			
Ca (g/kg)	6.85abS	6.85abS	7.60aS	7.48aS	7.13aS	6.46aS	6.30bS
Mg (g/kg)	3.60bcS	3.99aS	3.76abcS	3.90abS	3.51cS	3.53cS	3.75abcS
K (g/kg)	17.8aS	15.1bcdL	12.9dL	16.7abS	14.0cdL	5.1bcdL	16.3abcL
P (g/kg)	3.36aS	3.32ab*S	2.99b*S	2.91ab*S	2.87abS	3.13aS	3.40a*S
N (g/kg)	43.4cS	45.1bcS	47.1abcS	47.1abcS	43.8cS	48.2aS	46.9abS
Cu (mg/kg)	4.75cL	3.86cdL	3.15dL	7.25a*L	3.38cdL	7.13aL	6.50bL
Fe (mg/kg)	107.5aS	95.0aS	101.3aS	98.8aS	90.0aS	113.8aS	98.8S
Mn (mg/kg)	75.3cS	67.3cdS	56.4dS	76.3cS	65.4cdS	97.0bS	130.3aH
Zn (mg/kg)	33.8dS	47.4bcS	42.1cdS	56.9bH	50.6bcS	72.8aH	50.6bcS

abc:Values in rows not followed by the same letter are significantly different ($P < 0.05$)
* Mean values followed by an asterisk indicate significant differences in means between cultivars
L = low nutrient concentration; S = sufficient nutrient concentration. H = high nutrient concentration
L, S and H determined from crop nutrient interpretative values (Jones et al., 1991)

Copper was low in soybean tissue at all seven locations. The two cultivars of soybean ('Howard' and 'Deltapine 105') differed in the leaf concentrations of P in the wheat/maize, clover/maize, lupin/vegetables and lupin/maize crop rotation systems and Cu in the lupin/vegetables cropping system. The results also showed that with the exception of P and Mn in maize and Fe in soybean, there were significant differences in the levels of the other minerals in maize and soybean at the different locations. This is partly attributable to the different previous crop rotation systems of the experimental sites, which might have had different effects on the physicochemical and biological properties of the soil, resulting in variation in the nutrient availability to plants.

Table 2 shows the soil nutrient levels for the maize and soybean plots. Soil analysis is important to correct undetected insufficiencies. The range of soil nutrient levels for the corresponding deficient maize leaf elements were N, 335–548 mg/kg; Mg, 11.1–37.3 mg/kg; K, 13.1–66.1 mg/kg; Cu, 0.11–0.21 mg/kg and Zn, 0.59–1.55 mg/kg, and for soybean K, 19.7–98.4 mg/ kg and Cu, 0.10–0.20 mg/kg. In general, where these nutrients were low in the plant they were among the lowest values in the soil. Of all the soil analysis data only OM (organic matter) and Cu differed between the plots for the two cultivars and only at the clover/maize, rye/maize and vetch/maize crop rotation sites. It appears that adequate K fertilizer was applied to meet the needs of an average soybean crop. Low extractable K values in the soil indicated that fertilizer K must have been leached to below the soybean root zone.

Table 2

Kjeldahl nitrogen, pH, Wakley-Black organic matter and Mehlich 1 extractable nutrients after different previous crops for maize (Pioneer P3098) and soybean (Howard and Deltapine)

Nutrient	Previous crops						
	Fallow/ Peanut	Wheat/ Maize	Clover/ Maize	Lupin/ Vegetables	Rye/ Maize	Vetch/ Maize	Lupin/ Maize
				Maize			
N (mg/kg)	375a	435a	335a	475a	413a	548a	363a
pH	5.7a	5.9a	5.8a	5.7a	5.8a	5.3b	5.0b
OM (%)	1.42a	1.44a	1.25a	1.26a	1.59a	1.27a	1.37a
Ca (mg/kg)	237a	222a	324a	269a	273a	218a	209a
Mg (mg/kg)	24.8ab	30.0a	37.3a	31.3a	38.3a	25.0ab	11.1b
K (mg/kg)	66.1a	13.1b	16.3b	24.7b	22.7b	20.6b	15.1b
P (mg/kg)	64.3a	32.9c	51.1abc	40.3bc	45.8bc	54.8ab	36.5bc
Cu (mg/kg)	0.16ab	0.11b	0.12b	0.13b	0.19ab	0.20a	0.21a
Fe (mg/kg)	16.5a	7.8c	7.8c	8.9c	9.0c	10.5bc	12.3b
Mn (mg/kg)	4.3a	2.3a	3.9a	2.8a	2.6a	3.8a	2.8a
Zn (mg/kg)	0.59b	0.95ab	1.55a	1.42a	1.07ab	1.28a	0.57b
				Soybean			
N (mg/kg)	348b	435a	421ab	455a	381b	481a	390b
pH	5.9a	6.0a	6.1a	5.9a	6.0a	5.6b	5.4b
OM (%)	1.26b	1.16bc	1.15bc	1.22b	1.29b	1.43a *	1.46a
Ca (mg/kg)	243c	214c	387a	288bc	358ab	240c	114d
Mg (mg/kg)	29.6b	32.0b	44.0a	40.4a	43.9a	25.9b	13.7c
K (mg/kg)	98.4a	19.7b	32.7b	32.6b	28.1b	26.8b	22.5b
P (mg/kg)	61.2a	29.8d	61.6a	40.0c	54.8ab	55.8ab	43.5abc
Cu (mg/kg)	0.16b	0.10c	0.14bc	0.16b	0.20a *	0.15b	0.16b
Fe (mg/kg)	14.8a	7.7c	7.8c	8.6c	10.3bc	9.3bc	11.9b
Mn (mg/kg)	3.2a	2.2a	2.7ab	2.7ab	2.6ab	1.25b	2.7ab
Zn (mg/kg)	0.46e	0.80d	1.63a	1.16bc	1.16bc	1.25b	0.87cd

abc. Values in rows not followed by the same letter are significantly different ($P < 0.05$)

* Mean values followed by an asterisk indicate significant differences between cultivars.

Correlations between plant nutrients and soil pH, nutrients and organic matter for maize and soybean are shown in Tables 3 and 4, respectively. The concentrations of Mg, K and P in maize were positively correlated (r= 0.68, 0.59, 0.41, respectively) ($P < 0.01$) with the corresponding soil minerals. The concentrations of Ca, K and N in soybean were also positively correlated (r = 0.70, 0.41, 0.32 respectively) ($P < 0.01$) with the corresponding soil minerals. The nutrient insufficiency observed in maize and soybean for some minerals was therefore due to their low status in the soil during the sampling period. The correlation of plant minerals with pH showed that the concentrations of Cu and Mn in the ear leaf of maize were negatively correlated (r = –0.47, –0.7 respectively) ($P < 0.01$) with pH. The concentrations of Cu and Mn in soybean were also negatively correlated (r = –0.572, –0.716, respectively) ($P < 0.01$) with soil pH. The pH of the substrate influences plant concentrations primarily by affecting the availability of the nutrients. The concentrations of Fe, Mn and Zn in soybean tops were found to be negatively correlated with soil pH as the pH increased from 5.1 to 6.47 (Jones et al., 1991). Low soil pH, for example, will increase the availability of Cu, Fe, Mn and Zn, but decrease that of Mo.

Nitrogen fertilizer application frequency and amount should be evaluated in the light of its leaching potential in sandy soil. It appears that the four split applications currently recommended for maize were not enough to obtain sufficient N in the leaves. Splitting this N into 6 to 8 increments from planting to a short time past flowering would partly solve this problem. Also, increasing the total N by 25% may be needed to solve the problem. Since Mg values in the soil test were generally low, the use of dolomitic limestone as a limingagent, could help to alleviate the low levels of Ca and Mg in the leaf tissues of legume crops. Potassium appeared to be low in the leaf tissue of both crops, and to alleviate this problem K should be applied in three split applications from planting to one week before flowering. This should help avoid possible leaching from heavy rainfall. If large applications of K are recommended and soil Mg is low, Mg fertilizer should be applied so that the K does not antagonize the uptake of Mg and result in a Mg deficiency (Jones, 1974). For both maize and soybean Cu is low in the leaves and therefore foliar application of a Cu fertilizer is needed to solve this problem. The above recommendations should be implemented if these experiments are to be repeated in the future on Arredondo sandy soils.

The results of the study have implications for the production of maize and soybean in loamy sand to sandy soils, especially during periods of heavy rainfall, and more importantly under rain-fed agricultural production systems. The leaching potential of such soils would negate the benefits of applied nutrients, whose insufficiency could only be revealed by timely diagnostic analysis. The results also show that production systems that base fertilizer recommendations on leaf analysis, the soil test level, fertilizer and general growing conditions (including cropping history) stand to benefit from the primary practical field use of plant analysis today – to diagnose suspected elemental deficiencies (Jones et al., 1991).

Table 3
Correlation between plant nutrients and soil pH, minerals and organic matter for the maize hybrid Pioneer P3098

Nutrients	pH	Ca	Mg	K	P	N	Cu	Fe	Mn	Zn	OM
Ca	0.047	0.387*	0.418*	-0.474**	0.122	0.008	0.057	–0.540**	0.201	0.493**	–0.149
Mg	0.410*	0.543**	0.677**	–0.496**	0.063	–0.017	–0.200	–0.679**	0.114	0.510**	–0.043
K	–0.342+	–0.246	–0.378*	0.591**	0.269	0.078	0.359+	0.606**	0.121	–0.100	–0.015
P	0.207	0.360+	0.390*	0.029	0.411*	0.206	0.119	–0.090	0.260	0.104	0.054
N	–0.354+	–0.114	–0.143	0.340+	0.492**	0.070	0.264	0.420*	0.423*	0.156	–0.174
Cu	–0.471**	–0.319+	–0.339+	–0.110	–0.148	0.305	0.331+	0.100	–0.125	0.042	–0.259
Fe	–0.029	0.162	0.160	0.161	0.099	–0.100	0.298	0.047	0.057	0.159	0.071
Mn	–0.709	–0.801**	–0.744**	–0.073	–0.100	0.016	0.515**	0.463**	–0.030	–0.324+	–0.236
Zn	–0.198	–0.100	0.049	–0.655**	–0.362+	0.113	0.156	–0.509**	–0.352+	0.249	–0.207

+, *,** = Correlation significant at the 0.10 to 0.051, 0.05 to 0.010 and < 0.01 level of probability, respectively

Table 4
Correlation between plant nutrients and soil pH, minerals and organic matter for soybean

Nutrients	pH	Ca	Mg	K	P	N	Cu	Fe	Mn	Zn	OM
Ca	0.483**	0.698**	0.670**	0.122	0.175	0.146	–0.039	–0.122	0.089	0.259*	–0.169
Mg	0.050	0.020	0.113	–0.054	–0.200	0.166	–0.412**	–0.122	–0.119	–0.012	–0.117
K	–0.449**	–0.311**	–0.290*	0.405**	–0.017	0.037	0.093	0.599**	0.219+	–0.341**	0.053
P	–0.289*	–0.450**	–0.444**	0.150	–0.030	–0.142	–0.109	0.327**	0.115	–0.266*	0.126
N	–0.219+	0.034	–0.50	–0.310*	0.010	0.316**	–0.223+	–0.211	0.093	0.338**	0.123
Cu	–0.572**	–0.465**	–0.511**	–0.119	–0.183	0.268*	0.106	0.122	0.121	–0.043	0.287*
Fe	–0.086	0.125	0.087	0.202	0.288*	0.303*	–0.089	0.039	0.263*	0.150	–0.154
Mn	–0.716**	–0.644**	–0.772**	–0.205	–0.140	–0.025	0.122	0.271*	0.157	–0.135	0.320**
Zn	–0.360**	–0.170	–0.168	–0.481**	–0.076	0.357**	0.182	0.251+	0.018	–0.260*	0.221

+, *,** = Correlation significant at the 0.10 to 0.051, 0.05 to 0.010 and < 0.01 level of probability, respectively

Critical considerations for interpreting the results of plant and soil analysis include environmental effects, cultural practices, soil properties and other circumstances. While the current study focused on the effect of cropping history and variety, under given soil and management conditions, it nonetheless proposes that in future experiments on Arredondo sandy soils or other sandy soils, use should be made of data logging or tracking, a useful but little used technique for plant analysis utilization (Munson and Nelson, 1973). Jones et al. (1980) tracked the element contents of leaves and other plant parts in peanuts grown with irrigation on the sandy soils of South Georgia. Season to season changes in plant nutrition on sandy soils under irrigated or rain-fed conditions should be tracked for the same crop with the objective of regulating cultural practices and environmental effects so as to maintain plant elemental level within a sufficiency range. Corrective measures can be taken before an insufficiency occurs, which may otherwise lead to a reduction in yield and/or quality.

The current study revealed that the nutritional status of maize and soybean on Arredondo sandy soil was diversely affected by crop rotation (cropping history) and by the variety of soybean. The application of recommended fertilizer rates and sequence does not necessarily ensure plant elemental sufficiency status. Routine diagnostic leaf analysis is necessary to detect nutritional insufficiencies and thereby take corrective measures.

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COMPARATIVE STUDY ON DIFFERENT DRYING PROCEDURES AND FINISHING TREATMENTS FOR MAIZE

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Two different ways of maize finishing, one intensive (industrial) and one extensive, are compared in order to establish what losses occur and how great post-harvest losses are, as well as to determine the technological quality and hygiene status of maize as a raw material for industrial uses, involving processing either into animal feed or into human food (minor share).

Data show that the damage suffered in the silo-bin may be mechanical or thermal. Intensive maize kernel thermal treatment increases post-harvest losses. This problem could be eliminated by harvesting maize with a moisture content below 30% and by applying multiple stage drying, which in turn increases the cost of finishing treatments. Although drying and mechanical transport cause considerable physical kernel damage, the stored maize remains satisfactory in a sanitary sense for more than eight months, i.e. it is stable from a microbiological point of view and is suitable for further processing.

Sources of kernel damage in maize cribs are the long-term drying process required for maize on the cob, the immovable mass, poor crib aeration, i.e. faulty crib location. As regards sanitary status (especially microbiological aspects), maize can only be safely stored for 6 months if there is a severe winter with low temperatures.

Key words: maize, drying, quality, post-harvest losses

Introduction

In regions like Yugoslavia, the period when maize should be harvested is rarely as dry as could be desired. Modern technology enables maize harvesting to be completed within 10 to 15 days, which is very important because in that way fertile areas are liberated and can be prepared for winter crops. Very often, maize with a moisture content of over 30% is transported from state-owned farms to the receiving points. This high moisture content must be lowered to between 14 and 15%. Such raw material is usually dried in two stages. However, due to the low capacity of the dryers, which are usually a bottleneck in the chain from field to storehouse, higher temperatures are generally applied (drastic moisture lowering), which are certain to increase post-harvest losses (Bolling et al., 1973; Peplinski et al., 1975; Katić, 1971). After drying, maize kernels with a moisture content below 14% are stored in storehouses where air can be blown in. In this way the atmosphere in the space between the kernels is exchanged and the kernel temperature is regulated.

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In private farms maize is harvested on the cob at lower moisture content and is usually stored in maize cribs. During storing, the maize cobs do not undergo any thermal treatment. It is assumed that in the maize cribs the maize will dry to the moisture content optimal for storing through the process of desorption through the cob (Peplinski et al., 1975).

These two different ways of maize harvesting, that take place at various stages of seed maturity, are connected with two different treatments during storage which, at the same time, represent extremes in grain storing technology (Peplinski et al., 1975; Katić, 1971) and have a great influence on maize quality (Dorsey-Redding et al., 1991). As the quantities sold by private farmers are by no means negligible, it is very important to know the technological quality of this maize, too.

The objective of this paper is to compare two different ways of maize finishing, one intensive (industrial) and one extensive, to establish what losses occur and how great the post-harvest losses are, as well as to determine the technological quality and sanitary status of maize as a raw material for industrial uses, involving processing either into animal feed or into human food (minor share).

Materials and methods

Sampling was done in the dryer next to the maize silo-bin in the receiving hoppers, successively from the beginning to the end of the harvest. In this way wet material was collected. Parallel sampling at the dryer outlet was carried out in order to obtain information on the material that would be stored. During storage sampling was repeated every 30 days.

Sampling in maize cribs was first done immediately after the harvest, and repeated every 30 days during storage. Samples were taken from the silo-bins and maize cribs at the same time.

The moist kernels collected at the dryer inlet and kernels shelled from the cobs taken from the maize cribs were dried in the laboratory.

After being optimally stabilized for storing in the laboratory by drying in a thin layer at a temperature below 45°C, the samples were successively analysed in the same order as they had been collected.

First of all, the moisture content of the maize was determined, after which the 1000 kernel weight, test wight and content of impurities were also determined according to the Yugoslav standard E.B3.516. After cleaning, the ash, starch, protein, lipids and acidity were determined. In addition, the kernels were separated into anatomic parts by hand, so that the lipids and acidity could be determined separately in the endosperm and the germ.

Besides chemical analyses, the sanitary status of the maize was checked through microbiological analyses (total number of moulds, yeasts, aerobic mesophilic bacteria and aerobic mesophilic spore-forming bacteria). The microbiological analyses were done according to Yugoslav regulations. Biological activity was investigated by analysing seed germination and germination vigour. The physical appearance of the kernel was examined using a magnifying glass capable of magnifiying 5 or 15 times.

Statistical analysis was carried out according to Hadzivuković (1979).

Results and discussion

The data were grouped according to the type of analysis and the sampling period. This means that each data group was compared to the zero day, i.e. the first sampling date, then according to the sampling rhythm, which was 30 days.

Neither sample homogeneity in each probe, nor corresponding homogeneity relative to the overall mass could be achieved, because the raw material was tested on the macro scale of regular storage.

Kernel moisture

Moisture content, measured immediately after harvest and then during drying and storing, fluctuated considerably, giving the first warning that fluctuations beyond the limits of analytical error could be expected for this procedure of determination.

During a period of one month (from 12th November to 12th December) maize with a moisture content between 28 and 37% was delivered to the silo-bin. At the end of October maize on the cob with a moisture content between 24 and 29.9% was stored in the maize cribs (Table 1).

Table 1
Maize moisture content at delivery*

Drier	Grain moisture content (%)**	Private maize crib	Grain moisture content (%)***
I	36.6	I	29.9
II	35.0	II	28.7
III	34.0	III	24.0
IV	31.0		
V	30.0		
VI	28.5		

*Maize received in various periods (30 days); **After harvest, maize shelled in the field was transported to the drier; ***After harvest, maize on the cob was put into maize cribs and later shelled in the laboratory

After drying in two stages, the excess water was removed and the kernels had a moisture content of 14 to 15%. Such maize was stored in silo-bins where its commercial and technological quality was checked over a 160-day period. During this time, when air was periodically blown in, the moisture content fluctuated around 13%.

At the same time, in the maize cribs the moisture from the kernels migrated into the cob during the first month, thus lowering the moisture content to about 21% on average; over the next 30 days, no changes were observed (this was the coldest winter period). During the first days of spring, there was a sudden drop in moisture content. Five months after harvesting maize on the cob had naturally dried to a moisture content of 12.5 to 13%.

Physical properties of kernels

In the case of maize stored on the cob in maize cribs, the 1000 kernel weight varied significantly during storage, though at the beginning (during the first two months) no loss of moisture was observed. This was the maturing stage (Bekrić, 1990).

For samples stored in silo-bins, the 1000 kernel weight was higher both at the beginning and after two months of storage than that of maize stored in maize cribs. This difference is understandable because natural moisture diffusion from the kernel into the cob is very slow during winter or does not occur at all. During further storage this difference was still present in some samples but disappeared in others. One possible reason for the greater data fluctuation was unequal sampling, because it was very difficult to take truly average samples from an overloaded crib 2.2 m high, 8 m long and 1.2–2.0 m wide.

Test weight is not very important as an indicator though it shows up the difference between maize stored in silo-bins, which underwent a severe drying regime, and naturally dried maize on the cob stored in maize cribs.

At the very beginning, on zero day, the type of impurities depended to a great extent on the harvesting procedure, i.e. whether the maize was harvested as kernels or as maize on the cob (Bekrić, 1990; Table 2). This is confirmed by the high F-distribution values for total impurities regardless of the finishing treatment, and by the low F-distribution values for the content of impurities in maize dried on the cob.

The maize fed into the dryer contained about 8% broken kernels after combine harvesting, while after drying the content of broken kernels increased to 12%. During storage, because of manipulation and aeration, this figure increased again to 19.5%, but simultaneously the quantity of material passing through a screen with 1 mm holes was reduced and the quantity of material passing through a 5 mm screen increased slightly.

Two fractions, material passing through a 5 mm screen and broken kernels, which increased during the first four months of storage in silo-bins to as much as 20%, caused a great problem, which could be lessened by improving the drying procedure and the inner transportation in the storehouse. During the remainder of the storage period the share of these fractions remained fairly constant.

For maize stored on the cob in maize cribs, a steady increase in the ratio of musty kernels was observed, particularly after two months of storage.

Organoleptic inspection of the kernels mainly involved visual examination in order to obtain information about the type and size of physical damage.

On average, maize kernel damage (which comprises cracked corn) could be divided into 7 catergories (Thompson and Foster, 1968): separated germ, separated hull, open germ, back, side, front and net cracking (Fig. 1).

From the data in Table 3 it can be seen that back and side cracking are constantly present in high proportions, while the amount of front and net cracking and of separated parts varies. This is proof that the kernels fall to pieces during manipulation. Analyses on cobs stored in maize cribs (Table 2) showed a small proportion of cracking and damaged germs and the distinct appearance of moulds, particularly in maize crib number 2 (Table 4).

Microbiology of maize

The presence of mould on maize kernels stored in maize cribs, detected during the organoleptic inspection of the maize, was confirmed by microbiological analysis (Table 5).

Table 2
Content of impurities during storage

Crib/silo	Material passing through holes of		Broken kernels (%)	Damaged kernels (%)	Shrunken kernels (%)	Total impurities (%)
	1 mm (%)	5 mm (%)				
0 day						
Crib 1	0.07	0.03	0.30	–	–	0.41
Crib 2	0.16	0.86	0.36	–	–	1.58
Crib 3	0.06	–	–	–	0.08	0.12
Silo-bin	2.50	0.57	8.24	0.92	0.10	12.33
60 days						
Crib 1	0.07	0.04	0.30	1.00	–	1.41
Crib 2	0.20	0.90	0.40	0.60	–	2.10
Crib 3	0.06	–	–	–	0.08	0.14
Silo-bin	0.14	1.24	17.40	1.15	0.19	20.12
120 days						
Crib 1	0.07	0.05	0.40	1.00	–	1.52
Crib 2	0.20	0.50	0.40	3.60	–	4.70
Crib 3	0.06	–	–	–	–	0.06
Silo-bin	0.15	1.75	19.50	0.90	0.10	22.40
240 days						
Crib 1	0.07	0.05	0.40	1.00	–	1.52
Crib 2	0.20	0.50	0.40	6.00	–	7.10
Crib 3	0.06	–	0.04	–	–	0.46
Silo-bin	0.20	2.00	17.50	1.80	0.20	21.70

Statistics: F-distribution
1. Total impurities during storage
– calculated: 11.05
– theoretical (probability 0.05, degrees of freedom $r_1 = 4$, $r_2 = 16$): 3.49
2. Total impurities during storage in maize cribs
– calculated: 1.29
– theoretical (probability 0.05, degrees of freedom $r_1 = 3$, $r_2 = 12$): 4.26

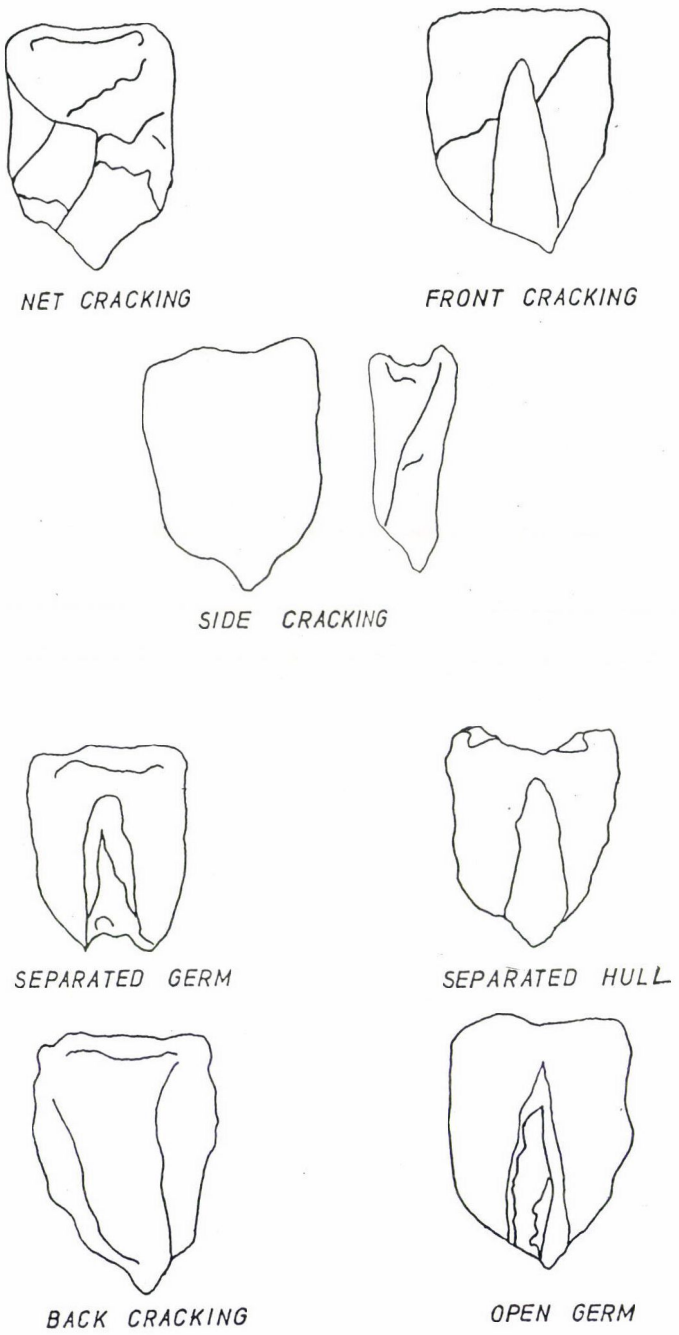

Fig. 1. Maize kernel damages

Table 3
Presence of different types of damage in the total mass of damaged kernels (%)

Damage type	0 day	30th day	60th day	120th day
Separated germ	5	1	6	1
Separated hull	12	16	18	12
Back cracking	40	34	38	34
Side cracking	17	19	20	28
Front cracking	12	14	0	0
Net cracking	9	13	0	22
Open germ	5	2	18	3

Microbiological analyses show that at the beginning, on zero day, dried kernels in silo-bins had a significantly smaller total number of moulds (0.1×10^3) than maize stored on the cob in maize cribs (0.2×10^3 to 4.0×10^3).

After only one month of storage, kernels from maize cribs had so many moulds that, with the exception of kernels from maize crib 3, they were not safe from a sanitary pont of view, making it dangerous to use them as animal feed, in the form of meal, or to store them further (Sietz et al., 1982).

The extremely severe weather in January and February in the year of investigation lowered the extent of mould infection in two of the maize cribs, but in maize crib 1 even the cold did not help. The weather conditions and the specific characteristics of the crib influenced the total number of yeasts, which ranged from 0 to 2.0×10^3 on zero day to 0 to 6.0×10^3 after four months of storage. The total number of bacteria did not change during storage in the silo-bin, but a significant increase was observed in the maize cribs, particularly in cribs 1 and 2. Aerobic mesophilic spore-forming bacteria were present in maize stored in both maize cribs and the silo-bin and their number ranged from 0 to 3.4×10^4. The data show that material stored in maize cribs further decays during storage and becomes useless for nutritional purposes.

Table 4
Damage such as cracking or colonies of moulds in maize stored in maize cribs

Maize crib	Cracking (%)	Colonies of mould (%)
0 day		
Crib 1	–	–
Crib 2	2	–
Crib 3	1	–
60 days		
Crib 1	1	3
Crib 2	2	2
Crib 3	2	–
120 days		
Crib 1	–	3
Crib 2	5	6
Crib 3	5	2

Table 5
Microbiological quality of maize kernels

Storage time and type of storage	Total number of moulds/g	Total number of yeasts/g	Total number of bacteria	Aerobic mesophilic spore-forming bacteria	Sanitary status
Crib 1					
0 day	4.0×10^3	2.0×10^3	3.0×10^3	0	safe
30 days	4.4×10^3	0	0.1×10^3	0	not safe
60 days	4.3×10^3	0	1.0×10^3	1.0×10^3	not safe
120 days	2.6×10^5	2.6×10^3	1.2×10^5	3.4×10^4	not safe
Crib 2					
0 day	1.0×10^3	0.1×10^3	1.0×10^3	1.0×10^3	safe
30 days	8.2×10^5	1.4×10^4	6.0×10^4	1.0×10^3	not safe
60 days	9.4×10^4	0	1.0×10^3	1.0×10^3	not safe
120 days	1.4×10^4	0	1.0×10^3	1.0×10^3	not safe
Crib 3					
0 day	0.2×10^3	0	1.0×10^3	1.0×10^3	safe
30 days	1.5×10^4	0	1.0×10^3	0	safe
60 days	2.9×10^4	0	1.0×10^3	0	safe
120 days	6.0×10^3	6.0×10^3	1.0×10^4	2.0×10^3	not safe
Silo-bin					
0 day	0.3×10^3	0.1×10^3	1.0×10^3	0	safe
30 days	2.0×10^3	0	1.0×10^3	1.0×10^3	safe
60 days	7.0×10^3	0	1.0×10^3	2.0×10^3	safe
120 days	8.0×10^3	0	1.0×10^3	1.0×10^3	safe

Although physically the material stored in a silo-bin represented a good basis for the development of microorganisms, because of the severe treatment, it remained exceptionally sound from the microbiological point of view (Table 5).

Biology of kernels

The reproduction ability of kernels from maize cribs was stabilized during the first month and they exhibited an exceptionally good germination percentage, though the germination vigour was smaller. Artificially dried kernels, however, had substantially decreased reproduction ability; the germination percentage and germination vigour prove that this raw material is inactivated in a biological sense (Table 6). The statistical data confirm that the high F-distribution value for germination is caused by low values for germination in artificially dried corn.

The germination and germination vigour data provide a better explanation of the damage. Damage to maize on the cob caused by the action of microorganisms decreases the germination vigour, while intensive drying, or drying at insufficiently controlled temperatures decrease to a minimum the biological properties of kernels stored in silo-bins, thus signalling that the technological properties are also reduced (decreased yield of maize products). The statistical data indicate that the germination vigour depends on the drying method.

Table 6
Maize germination and germination vigour

Storage time and type of storage	Germination (%)	Germination vigour (%)
Maize crib 1		
0 day	100	66
30th day	100	65
60th day	100	60
120th day	100	48
Maize crib 2		
0 day	100	72
30th day	100	55
60th day	100	62
120th day	100	58
Maize crib 3		
0 day	100	64
30th day	100	53
60th day	100	53
120th day	100	92
Silo-bin		
0 day	14	8
30th day	16	12
60th day	19	15
120th day	45	20
Statistics:		
F-distribution		
1. calculated	27.9	4.89
2. theoretical*	3.49	3.49

*probability 0.05; degrees of freedom r_1=4, r_2=16

Chemical properties of kernels

The chemical properties (ash, protein and lipids) of the maize stored in the three maize cribs and in the silo-bin showed little change, meaning that the raw material suffered no technological damage (Table 7).

Two chemical constituents should nevertheless be taken into consideration (Dorsey-Redding et al., 1971; Bekrić, 1990). First, the starch content, which showed variations depending upon the samples, i.e. there was an unequal kernel mass caused by maize shelling in comparison with fairly uniform material from the silo-bin. Second, there was a higher content of lipids in the endosperm of kernels from the silo-bin than in kernels stored in maize cribs. This was due to the drying process, which is the second key point for the elimination of undesirable influences on the raw material. Germ lipid content is not important, but its acidity is. Statistical data confirm these differences.

Table 7
Chemical composition of maize

Storage time and type of storage	Content (% d.m.) of						Acidity of germ lipids
	Ash	Starch	Protein	Lipids in			
				Whole kernel	Endosperm	Germ	
Crib 1							
0 day	1.35	72.2	9.3	3.7	0.18	29.7	2.47
30 days	1.34	72.8	9.1	3.8	0.16	30.1	2.41
60 days	1.31	72.6	9.0	3.9	0.18	30.2	2.30
120 days	1.36	72.8	9.0	3.9	0.16	31.0	2.40
Crib 2							
0 day	1.32	75.2	9.2	3.7	0.16	32.6	12.80
30 days	1.31	74.3	9.2	3.7	0.18	32.0	12.72
60 days	1.29	74.2	9.2	3.7	0.20	29.6	12.40
120 days	1.32	75.0	9.2	3.7	0.18	30.6	12.62
Crib 3							
0 day	1.29	75.8	9.2	3.7	0.31	30.6	2.60
30 days	1.34	75.8	9.3	3.9	0.29	32.0	2.90
60 days	1.36	75.6	9.3	4.0	0.26	30.7	2.20
120 days	1.35	74.2	9.2	3.9	0.29	32.4	2.80
Silo-bin							
0 day	1.27	72.5	9.2	4.0	0.36	30.9	20.50
30 days	1.29	72.8	9.0	4.0	0.32	30.2	12.50
60 days	1.35	73.0	9.2	4.1	0.31	29.9	18.20
120 days	1.32	72.5	9.0	4.0	0.32	29.2	20.20
Statistics:							
F-distribution							
1. calculated	1.00	7.85	0.64	2.75	8.00	0.56	16.9
2. theoretical*	3.49	3.49	3.49	3.49	3.49	3.49	3.49

*probability 0.05; degrees of freedom $r_1=4$ $r_2=16$

Conclusions

The kernels suffered both mechanical and thermal damage in the silo-bin. Intensive thermal treatment increases post-harvest losses. This problem could be eliminated by harvesting maize with a moisture content of below 30% and by applying multiple stage drying, though this increases the cost of finishing treatments. In addition, the kernels are transported on chain conveyors, so mechanical damage is inevitable, but this could, in some measure, be decreased by enlarging the load in the transporters, which in turn would decrease the speed and should reduce the influence of kernel impacts. Though considerable physical damage is caused by the dryer and by mechanical transport, the stored

maize remains safe in the sanitary sense for more than eight months, i.e. it is stable in terms of microbiology. Chemical analyses show that there is no nutritive deterioration, which is very important for any kind of further processing. If physically damaged maize is processed, however, the yield of final products is certain to be decreased.

The sources of kernel damage in maize cribs are the long-term drying process of maize on the cob, the immovable mass, poor crib aeration i.e. faulty location of the crib in the yard. As regards sanitary considerations (microbiology, above all), even in the case of a severe winter with low temperatures, like that experienced when these investigations were carried out, the maize can only be regarded as safe for 6 months.

The maize from maize cribs could be stabilized again after deshelling and drying, but this augments the costs. It is a very important fact, however, that considerable quantities of maize harvested on private farms could be preserved if, after winter storage in maize cribs, it was dried to a moisture content of 13 to 14% in an appropriate drier and stored in a silo-bin. Drying as a means of post-harvest treatment, is very often inevitable in order to preserve the usefulness of the raw material.

The data thus show that, though the weather conditions were favourable, maize stored on the cob should be treated in early spring because of the very intensive action of microorganisms, which could lead to complete deterioration of the maize.

Maize which is intensively dried after post-harvest treatment undergoes substantial damage, but remains suitable in the health, biology and technological sense for further processing.

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Review

MOLECULAR PLANT IMPROVEMENT FOR RESISTANCE TO BACTERIAL PATHOGENS

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The molecular improvement of crop plants for resistance to pathogenic bacteria combines reliable *in vitro* regeneration techniques with recently developed efficient genetic transformation methods. This combination provides the basis for the introduction and expression of foreign genes in the plant genome, that code for a wide range of antibacterial proteins or initiate a number of defence pathways. The author reviews some of these strategies including (i) various heterologous proteins that interact with the bacterial membrane, (ii) the inactivation of bacterial non-host-specific toxins, (iii) the potential role of polysaccharides in the control of bacterial pathogenesis, and (iv) the interaction of bacterial avirulence genes and plant resistance genes. The need for the integration of these approaches with methods applied in classical resistance breeding is also stressed.

Key words: bacterial pathogens, molecular breeding, resistance

Introduction

Molecular plant improvement is becoming the widely-used term for the non-conventional breeding of crop plants using a range of molecular biology techniques. This range includes the identification, isolation and characterisation of novel genes with agronomic importance and their introduction into cultivated plant varieties via the combination of *in vitro* regeneration and genetic transformation (also called the transgenic approach). The expression of these foreign genes may result in increased abiotic stress tolerance, resistance to viral, bacterial or fungal pathogens or pests, or may provide improved nutritional, physiological or storage quality. However, molecular improvement should not be regarded as just an alternative way of classical breeding: it is rather a complementary tool, because classical and molecular breeding rely on each other in the initial and final phases of research, *i.e.* during the identification of genes of interest and the field testing of the transgenic cultivar (or further improving it) (see also Michelmore, 1995).

In this paper, background information and practical results are reviewed in the area of resistance to bacterial pathogens where molecular improvement has great prospects.

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Molecular breeding of crops resistant to bacterial pathogens

At present, the development of bacterial pathogen-resistant crops appears to have lagged behind that of other pathogen-resistant crops which contrasts with the extensive knowledge about plant-bacterial interactions. The involvement of extracellular bacterial polysaccharides in plant pathogenesis is becoming more and more documented (reviewed by Denny, 1995). Non-host-specific toxins are known to be the major factors in bacterial virulence (reviewed by Mitchell, 1984) and may play an important role in pathogenesis and symptom development (reviewed by Gross, 1991). Also, the first avirulence genes were cloned from bacteria (Staskawicz et al., 1984) and the very first plant resistance genes defined and cloned in tomato (Martin et al., 1993; Salmeron et al., 1996) and *Arabidopsis* (Bent et al., 1994; Mindrinos et al., 1994; Grant et al., 1995) conferred specific resistance to *Pseudomonas syringae* strains with corresponding avirulence genes. Thus, at present, plant-bacterial interactions provide the best model systems for studying gene-for-gene relationships and the molecular basis of plant disease resistance. In addition, a number of animal and plant peptides or proteins with antibacterial activity have also been described. Below, each of these approaches will be examined for its potential to control bacterial plant pathogenesis.

Expression of heterologous antibacterial peptides or proteins in transgenic plants

Independently from the original source organism, most of the antibacterial peptides interact with the bacterial membrane by forming transient ion channels and pores, by blocking the membrane's own ion channels or by inhibiting the synthesis of membrane proteins. Some of these peptides are active in a wide range of organisms including viruses and various eukaryotes while others have specific activity only in a group of bacteria, *e.g.* in the Gram-negatives.

Probably the best-known antibacterial peptides of **insect** origin are the cecropins, which accumulate in the haemolymph of giant silkmoth (*Hyalophora cecropia*), silkworm (*Bombyx mori*) and *Drosophila* as a response to infection. These short linear polypeptides (31–39 amino acids) interact with the outer phospholipid membranes of both Gram-negative and Gram-positive bacteria and modify them by forming a large number of transient ion channels (Durell et al., 1992). Cecropins are active *in vitro* against a wide range of phytopathogenic bacteria (Nordeen et al., 1992; Mills and Hammerschlag, 1993) and, therefore, have been considered as potential candidates to protect plants against bacterial pathogens. Apart from an early positive report (Jaynes et al., 1993), accumulating evidence has shown that the expression of cecropins *in planta* does not result in resistance to bacterial pathogens due to the low expression of the peptides (Hightower et al., 1994) and to early proteolysis by endogenous

proteases in transgenic plants (Mills et al., 1994; Florack et al., 1995). However, Owens and Heutte (1997) found that a synthetic mutant but active form of cecropin B (MB39) with one amino acid substitution became on average three times more resistant to degradation by various endogenous peptidases and proteases from the intercellular fluid of plant leaves. Low expression in plants of this and other foreign proteins from lower organisms may also be due to a difference in codon usage (Perlak et al., 1991) and in potential regulatory sequences that determine intracellular localisation or intercellular secretion of the foreign protein. When the same mutant cecropin MB39 gene was fused to an α-amylase secretion signal and expressed under the control of a proteinase inhibitor gene promoter in transgenic tobacco plants (Huang et al., 1997), the infiltration of *P. syringae* pv. *tabaci* in the leaves resulted in no or delayed symptom development. In addition, Huang et al. (1996) introduced a different synthetic cecropin B gene into two japonica rice varieties and found it to be transcriptionally active in the transgenic plants, with some lines showing improved resistance to the bacterial blight and streak diseases of rice.

A group of similar insect defensins have also been purified from flesh fly (*Sarcophaga peregrina*), including three sarcotoxin families and the family of sapecins (reviewed by Natori, 1994). Sarcotoxins are active against a wide range of Gram-negative bacteria and the gene coding for sarcotoxin IA has been recently introduced into potato by *Agrobacterium*-mediated transformation (Galun et al., 1996). Sapecins are 40-residue polypeptides with six half-cystine residues essential for antibacterial activity, mainly against Gram-positive bacteria. Sapecins show a significant structural and functional similarity to charybdotoxin from scorpion venom by being able to bind to calcium-activated potassium ion channels (Yamada and Natori, 1991). New toxins with homology to the sapecin family, like royalisin from the royal jelly of honeybees (Fujiwara et al., 1990) or phormicin from *Phormia terranovae*, have also been isolated. These antimicrobial peptides have not yet been tested with phytopathogenic bacteria.

The same insects also synthesise another family of six 20-kDa antibacterial proteins, the attacins, in response to bacterial infection (Hultmark et al., 1983). Attacins alter the structure and permeability of prokaryotic membranes by inhibiting the synthesis of membrane proteins (Carlsson et al., 1991). Increased *in vitro* and greenhouse resistance to *Erwinia amylovora* by the expression of attacin E in transgenic apple plants was reported by Norelli et al. (1994). This work is continuing for the control of the fire blight disease in various apple cultivars (Borejsza-Wysocka et al., 1997), but the gene encoding attacin is used in combination with the bacteriophage T4 lysozyme gene (see below). Also, Chen and Kuehnle (1996) have demonstrated the expression of attacin in calli induced from transformed *Anthurium* plants.

A number of distinct, potentially interesting antibacterial polypeptides have been described in other insects including apidaecins from honeybees (Casteels et al., 1989) and moricin from silkworm (Hara and Yamakawa, 1995).

A family of antimicrobial peptides from a **lower invertebrate** are the tachyplesins first isolated from acid extracts of Japanese horseshoe crab (*Tachypleus tridentatus*) haemocytes. These strongly basic 2.3 kDa polypeptides (17–18 residues) with two disulphide bridges primarily inhibit the growth of both Gram-negative and Gram-positive bacteria by forming a complex with bacterial lipopolysaccharides (Nakamura et al., 1988) or with phospholipid membranes (Oishi et al., 1997). Very recently, Allefs et al. (1996) have fused the sequence encoding a synthetic tachyplesin I gene with that of the barley hordothionin signal peptide. A low expression of this chimaeric gene in three potato cultivars revealed slight inhibitory effects to *Erwinia carotovora* ssp. *atroseptica*. Curiously, tachyplesin was also found to be very effective in controlling the growth of bacteria that are typically found in vase water like *Bacillus*, *Enterobacter* and *Pseudomonas* spp. (Florack et al., 1996).

The expression of antibacterial proteins from **higher animals** in plants has scarcely been investigated. The best-known toxins which are isolated from frog skin (reviewed by Barra and Simmaco, 1995) such as the pore-forming magainins (reviewed by Bevins and Zasloff, 1990), bombinins (Simmaco et al., 1991), brevinins and esculentin (Simmaco et al., 1993; 1994), rugosins (Suzuki et al., 1995) and temporins (Simmaco et al., 1996) have not been expressed successfully in plants yet. Interestingly enough, human lactoferrin, originally found in milk, a member of a family of iron-binding glycoproteins, was reported to have antibacterial properties in transgenic plants. Mitra and Zhang (1994) observed that the introduction of a human lactoferrin cDNA into tobacco resulted in the expression of a truncated lactoferrin protein which exhibited enhanced activity against several pathovars of *P. syringae*, *Xanthomonas campestris* pv. *phaseoli*, and *Clavibacter flaccumfaciens* pv. *flaccumfaciens*. However, this observation may not be surprising since Bellamy et al. (1992) found that lactoferricin, an acid-pepsin cleavage product of lactoferrin, had activity against a broad range of bacteria.

Another source of antibacterial proteins has been lysozyme either from bacteriophages or from hen's eggs. These enzymes attack the murein layer of bacterial peptidoglycan resulting in cell-wall weakening and eventually leading to the lysis of both Gram-negative and Gram-positive bacteria. Hippe et al. (1989) reported that the expression of a bacteriophage T4 lysozyme with a plant signal peptide in transgenic tobacco plants was localised in the intercellular space. The application of this strategy in transgenic potato tubers led to increased resistance to infection by *E. carotovora* ssp. *atroseptica* in the greenhouse (Düring et al., 1993). A lysozyme gene from bacteriophage T7 has also been used to construct expression vectors for plant transformation (Huang et al., 1994). Initial experiments with hen lysozyme for bacterial resistance have met with little success (Trudel et al., 1992) due to a low level secretion of the lysozyme with its own signal peptide to the intercellular space. However, high extracellular secretion of hen lysozyme in transgenic tobacco resulted in growth inhibition of *Clavibacter michiganense* and *Micrococcus luteus* (Trudel et al.,

1995) in *in vitro* assays. Very recently, a synthetic human lysozyme gene has also been expressed in transgenic tobacco plants and found to inhibit the growth of *P. syringae* pv. *tabaci* in these plants (Nakajima et al., 1997).

Higher plants are also a rich source of various proteins with lysozyme activity, like a chitinase from cucumber (Métraux et al., 1989) or hevamine from *Hevea brasiliensis* (Jekel et al., 1991). However, these proteins have not been expressed so far in transgenic plants in order to control bacterial infections. The best-characterised plant antimicrobial proteins are thionins (Florack and Stiekema, 1994), including pseudothionins (Moreno et al., 1994), that have shown *in vitro* inhibition activity to a broad range of pathogenic bacteria (Molina et al., 1993a). Of these, correct expression of a barley α-thionin gene in transgenic tobacco enhanced resistance to two pathovars of *P. syringae* in *in vitro* tests (Carmona et al., 1993), while synthetic hordothionin genes were not secreted into the intercellular space in transgenic tobacco plants (Florack et al., 1994). Unfortunately, most thionins can be toxic to animal (Carrasco et al., 1981) and plant cells (Reimann-Philipp et al., 1989), and thus may not be ideal for conferring resistance in transgenic plants. Plant defensins from various plant seeds (Duvick et al., 1992; reviewed by Broekaert et al., 1995) or lipid transfer proteins (Molina et al., 1993b; Segura et al., 1993; reviewed by García-Olmedo et al., 1995) may be better candidates for use against some bacterial pathogens. For instance, recently Molina and García-Olmedo (1997) found that when they expressed a barley lipid transfer protein cDNA in transgenic tobacco and *Arabidopsis* the percentage of necrotic lesions, the average size of these lesions and the total lesion area were significantly reduced in both species after inoculations with compatible pathovars of *P. syringae*.

Many of the toxic polypeptides mentioned above are interesting for the control of bacterial pathogens in plants and it may be worth exploring their effects on these bacteria in *in vitro* assays as a preliminary test prior to their potential use in transgenic plants. In addition, more efficient synthetic compounds designed by combining different protein domains responsible for toxicity to bacteria (Powell et al., 1995; Desnottes, 1996) could also be tested.

Finally, a promising strategy for bacterial control could be the expression of monoclonal antibodies that target specific bacterial pathogenicity factors in transgenic plants (Düring, 1996). The specific binding of antibodies to one or more bacterial factors, such as secreted lytic enzymes or extracellular proteins, is expected to compromise bacterial pathogenicity. The feasibility of this concept has already been suggested for antiviral strategies. The success of this approach is based on two conditions: (i) efficient antibody production techniques, and (ii) the correct expression and secretion of antibodies in plants. Unlike the laborious hybridoma technology, the recently developed phage display technique (Scott and Smith, 1990; Clackson et al., 1991) will offer a more convenient and faster method to produce single-chain variable antibody fragments (scFv's) that is based on bacterial recombination and gene expression

techniques instead of large-scale work with live animals for immunisation. In addition, phage display allows the ready isolation of the gene encoding the desired antibody. On the other hand, both full-size as well as scFv antibodies have already been successfully expressed in transgenic plants for various applications (reviewed by Whitelam and Cockburn, 1996; Conrad and Fiedler, 1994). Here, the obvious advantage of recombinant scFv's to full-size antibodies lies in that only correct folding is required and not assembling of the different subunits.

Inactivation of bacterial toxins

Pathogenic organisms produce various toxins which can be classified as (i) host-specific toxins that account for the specificity of the host-pathogen interaction at the molecular level, and (ii) non-host-specific toxins that are able to affect a wider range of host and non-host organisms than the producing pathogens do (reviewed by Mitchell, 1984; Gross, 1991). From a practical point of view, only non-host-specific toxins can be considered for a broad resistance to bacteria because host-selective toxins are too specific and they have been identified so far only in fungi. In addition, pathogens must have developed a mechanism for self-protection to their own non-host-specific toxins which can then be "borrowed" and transferred into the host.

Similarly to the inactivation of antibiotics, two major strategies have been adopted by bacteria to protect themselves from their own toxin: (i) resistance based on the production of insensitive target enzymes (reviewed by Durbin and Langston-Unkefer, 1988), or (ii) resistance by detoxifying enzymes (reviewed by De la Fuente-Martínez and Herrera-Estrella, 1993).

The best characterised bacterial toxins originate from various pathovars of *P. syringae* (Table 1). Consequently, the transgenic approach has been applied only to this family of toxins so far.

Table 1
List of characterised bacterial toxins in various pathovars of *Pseudomonas syringae*

Name	Pathovar	Biochemical target
Coronatine	*glycinea, tomato*	ethylene biosynthesis?
Phaseolotoxin	*phaseolicola*	ornithine carbamoyltransferase (OCTase)
Syringomycin	*syringae*	plasma membrane H^+-ATPase
Tabtoxin	*tabaci, coronafaciens*	glutamine synthetase
Tagetitoxin	*tagetis*	eukaryotic (plastid) RNA polymerase III

A typical example of insensitive target enzymes is ornithine carbamoyltransferase (OCTase), a key enzyme in the arginine and polyamine biosynthetic pathway in plastids, that is inhibited by phaseolotoxin, a tripeptide toxin. Phaseolotoxin-producing bacterial strains, however, synthesise a different OCTase that is resistant to the toxin and its derivatives (Mosqueda et al., 1990). When expression of this toxin-insensitive OCTase was targeted to chloroplasts in transgenic tobacco plants, *in vitro* and biological assays demonstrated the complementation of the sensitive endogenous enzyme by toxin resistance and the hypersensitive reaction of transgenic plants to *P. syringae* pv. *phaseolicola* (De la Fuente-Martínez et al., 1992; Hatziloukas and Panopoulos, 1992).

Tabtoxin, another phytotoxic dipeptide from *P. syringae* pv. *tabaci,* inhibits the target enzyme glutamine synthetase and causes chlorotic symptoms (wildfire disease) in tobacco. The toxin-producing strains are insensitive to the toxin due to inactivation by an acetyltransferase enzyme encoded by the *ttr* gene. Transgenic tobacco plants expressing this tabtoxin acetylase showed high levels of resistance to the purified toxin or to infection by *P. syringae* pv. *tabaci* (Anzai et al., 1989).

A more practical example apart from toxins of pseudomonads is albicidin, the major component of a family of phytotoxins and antibiotics produced by the xylem-invading bacterium *Xanthomonas albilineans* that specifically block prokaryotic DNA replication (Birch and Patil, 1985). Albicidin is involved in leaf scald disease development in sugarcane, causing chlorosis in infected host plants. Genes involved in various mechanisms of resistance to albicidin have been identified and cloned from heterologous bacteria such as *Klebsiella oxytoca* (Walker et al., 1988), *Escherichia coli* (Birch et al., 1990), *Alcaligenes denitrificans* (Basnayake and Birch, 1995) and *Pantoea dispersa* (syn. *Erwinia herbicola*) (Zhang and Birch, 1997). The presence of a certain level of N-terminal homology between some of these genes at the protein level suggests a common functional domain. The gene *albD* from *P. dispersa* has recently been found to encode an esterase enzyme that belongs to the serine hydrolases (Zhang and Birch, 1997). The AlbD enzyme irreversibly detoxifies albicidin and its expression in *X. albilineans* blocks albicidin production and diminishes symptom development in infected sugarcane. Therefore, this gene may be a useful candidate for transfer into the sugarcane genome in order to confer disease resistance.

Bacterial polysaccharides in plant pathogenesis

Many phytopathogenic or symbiotic bacteria, including several species of *Clavibacter*, *Erwinia*, *Pseudomonas*, *Rhizobium* and *Xanthomonas,* produce large amounts of extracellular polysaccharide (EPS) during growth and during pathogenesis (reviewed by Denny, 1995). EPSs have multiple functions and

appear to provide a selective advantage for bacteria during their epiphytic or saprophytic existence: they protect bacteria from desiccation, concentrate nutrients, and enhance attachment to surfaces. During pathogenesis, EPSs regulate and minimise interaction with plant cells, thereby reducing the effect of host defence responses (Király et al., 1997) and contact with toxic substances, while promoting colonisation. In addition, EPSs may play a primary role in the development of disease symptoms, *e.g.* wilting caused by the plugging of xylem vessels.

At least three gene clusters important for EPS biosynthesis are organised more or less similarly in various phytopathogenic bacteria. For instance, in *Pseudomonas solanacearum* an 18-kb *eps* operon with at least nine genes is responsible for the acidic component of EPSs (Huang and Schell, 1995), and the *ops* cluster that contains at least seven structural genes seems to be necessary for the nucleotide sugar components of both EPSs and lipopolysaccharides (Kao and Sequeira, 1991). Recently, Kao et al. (1994) identified the regulator gene *epsR* whose overexpression resulted in decreased EPS production and reduced virulence of *P. solanacearum*. Though the expression of the *eps* operon in *P. solanacearum* appears to be controlled by a complex regulatory network (Huang et al., 1995) which is environmentally responsive, it may be interesting to express either *epsR* or another mutated regulatory system like *vsrB/vsrC* in the apoplast of transgenic plants to see whether they affect *in planta* EPS production and the virulence of *P. solanacearum* or not.

An attractive way to understand the role of EPS in more depth and eventually increase disease resistance by reduced EPS production in phytopathogenic bacteria could be by using polysaccharide depolymerase enzymes. Hartung et al. (1986) described the isolation of a polysaccharide depolymerase gene from a bacteriophage of *Erwinia amylovora*. The corresponding enzyme when expressed in *Escherichia coli* lysed the EPS of *E. amylovora* indicating that correct expression of the gene in plants may be interesting for testing this approach to control bacterial diseases. It should be mentioned that numerous bacteriophages of plant pathogens were described in the past (Okabe and Goto, 1963), including phages of *P. solanacearum* (Hayward, 1964).

Bacterial avirulence genes and plant disease resistance genes

The gene-for-gene concept (Flor, 1971; reviewed by Keen, 1990) explains host-pathogen recognition events on the assumption that single plant disease resistance genes and corresponding single avirulence genes in the pathogen together determine if the plant-pathogen interaction will be compatible (resulting in infection and disease development) or incompatible (resulting in the initiation of hypersensitive defence response and disease resistance). Biological races of pathogens that contain a set of avirulence (*avr*) genes will therefore induce a specific pattern of compatible and incompatible reactions

when inoculated on a collection of host plant cultivars. This pattern will depend on the presence or lack of complementarity between the *avr* genes of the pathogen and the disease resistance genes of host cultivars. Most models following this concept propose that pathogens are subjected to a kind of plant surveillance system which is similar to a receptor-ligand interaction in that the resistant plant recognises a pathogen-produced elicitor, a direct or indirect product of an *avr* gene (Gabriel and Rolfe, 1990). It should be noted, however, that when matching resistance genes are absent (*i.e.* in compatible interactions) the prevalent function of many *avr* genes may be to contribute to the pathogen's virulence (Dangl, 1994). In addition, it has been found that a gene cluster called *hrp* (**h**ypersensitive **r**esponse and **p**athogenicity) determines the expression of *avr* genes (Huynh et al., 1989) in a wide range of phytopathogenic bacteria. *hrp* genes probably control the secretion and transport of *avr* gene products outside bacterial cells, since at least eight of the 20 known *hrp* genes show remarkable homology to proteins involved either in protein secretion pathways (Van Gijsegem et al., 1993; Bogdanove et al., 1996) or in the flagellum biogenesis (Rosqvist et al., 1995) of animal pathogenic bacteria. Moreover, Pirhonen et al. (1996) demonstrated that non-pathogenic *E. coli* cells transformed with a functional *hrp* gene cluster when co-ordinately expressing the *avrB* gene were able to trigger a genotype-specific hypersensitive response (HR). The *hrp* gene cluster itself encodes and determines the secretion of a protein called harpin which is also able to elicit HR when infiltrated into plant leaves (He et al., 1993).

During the last decade over 30 bacterial *avr* genes have been cloned, mainly from strains of *P. syringae* and *X. campestris*, and more recently several plant disease resistance genes corresponding to some of these *avr* genes have also been cloned and characterised at a molecular level. However, little is known about the gene products, their function and the mechanism by which they act during incompatible plant-bacterial interactions. So far only the *avrD* gene product of *P. syringae* has been shown to function as an enzyme that is involved in the synthesis of syringolides which are able to elicit HR (Keen et al., 1990). Very recent results indicate that the *avrBs2* gene product of *X. campestris* may also be an enzyme (Swords et al., 1996) and may act within plant cells (Yang and Gabriel, 1995; Gopalan et al., 1996). The predicted sequence of the AvrBs2 protein shows similarity to enzymes involved in the synthesis or hydrolysis of phosphodiester linkages like agrocinopine synthase from *Agrobacterium tumefaciens*. All the indirect evidence that Avr proteins may be delivered into plant cells via the Hrp-system seems to indicate that Avr proteins are primarily involved in bacterial pathogenesis rather than merely controlling genotype-specific HR elicitation (Collmer, 1996).

Though it would be tempting to express an *avr* (or a harpin) gene in transgenic plants in order to create bacterial disease resistance by HR elicitation, there are a number of arguments against this approach for immediate practical use. First, as seen before, *avr* genes determine race-specific plant recognition

events; thus, a whole set of genes would be necessary to provide protection against a wide range of pathogenic races. Second, it is possible that mutations would abolish the capacity of an *avr* gene to induce HR, as already reported for a fungal avirulence gene (Joosten et al., 1994). Third, since the function of most *avr* genes is not yet clear, the expression of an *avr* transgene alone might not induce HR, as already observed when bacteria harbouring various *avr* genes were infiltrated into plant leaves (Dangl, 1994). If *avr* genes are involved in some aspects of bacterial virulence, their expression in plants would represent a risky way of protecting plants from bacterial attack. It should be mentioned, however, that very recently Gopalan et al. (1996) have shown that constitutive ectopic expression of the *avrB* gene of *P. syringae* in *Arabidopsis* plants with the matching *RPM1* resistance gene does result in HR. Finally, the expression of *avr* genes in plants would require very tightly regulated (preferably pathogen-inducible) promoters, otherwise, background or leaky expression of the *avr* transgene could lead to an uncontrolled HR (Michelmore, 1995).

Five plant resistance genes to bacterial diseases have been cloned to date (Martin et al., 1993; Bent et al., 1994; Mindrinos et al., 1994; Grant et al., 1995; Song et al., 1995; Salmeron et al., 1996) and all of them have been isolated by map-based cloning which includes genetic localisation, marker saturation in the region localised, isolation of large genomic (BAC or YAC) clones, identification of cDNAs, and finally complementation. Though different classes of resistance genes may exist (Michelmore, 1995), surprisingly these genes have shown a structure related not only to each other but also to other plant resistance genes (Dangl, 1995). Some of their common features are (i) a variable number of leucine-rich repeats which indicate a function for protein-protein interactions (Kobe and Deisenhofer, 1994), and (ii) a P-loop motif with a common nucleotide-binding site (NBS) in diverse proteins with ATP/GTP binding activity (Saraste et al., 1990), suggesting that the binding of these nucleotide triphosphates to the proteins is essential for their functioning (Traut, 1994). Basically, all these genes seem to encode components of receptor systems and probably form part of a signal transduction pathway which in the end triggers an array of general defence reactions such as reinforcement of the cell wall, synthesis of phytoalexins and oxidation of phenolic compounds, activation of defence-related genes and HR. The fact that the majority of the plant disease resistance genes isolated so far belong to the receptor-related class may suggest that many of the classical resistance genes will fall into this category (Michelmore, 1995). Furthermore, while significant sequence similarities were found among the different disease resistance genes this is not the case for *avr* genes. These observations may reflect a common or similar recognition mechanism on the plant side of various invading factors which, on the other hand, are likely to be different for each pathosystem (Dangl, 1994). This is also supported by recent results when the first cloned resistance gene *Pto* (a tomato resistance gene against *P. syringae* pv. *tomato* carrying *avrPto*) was transferred to and found to function both in *Nicotiana tabacum* (Thilmony et al., 1995) and

N. benthamiana (Rommens et al., 1995), which suggests that disease resistance functions are indeed conserved in a wide range of plant species.

So far, nothing was known on the mechanism used by resistance genes to trigger the defence reactions listed above. However, very recently Zhou et al. (1997) identified several classes of cDNAs encoding proteins that physically and specifically interact with the Pto protein. More interestingly, the sequence of these proteins showed significant homology to a wide range of transcription factors, some of them with ability to bind to a core sequence (the PR box) present in the promoters of a large number of plant defence genes. Zhou et al. (1997) also showed that *Pto-avrPto* recognition indeed correlated with the early induction and increased expression of pathogenesis-related (PR) genes that contained the PR box, providing indirect evidence for a direct connection between a disease resistance gene and the specific activation of plant defence genes. Also, Zhou et al. (1995) have previously shown that another Pto interacting protein, Pti1, is involved in the induction of HR. Thus, at least two pathways of plant defence response can now be directly linked to a particular plant disease resistance gene.

Another important part of the defence mechanism is a rapid and transient release of different active oxygen species (AOS), also called as oxidative burst, such as the superoxide anion radical (O_2^-), hydroxyl radical (OH^-) and hydrogen peroxide (H_2O_2). Of these AOS, the production of hydrogen peroxide appears to be an early event that is involved in directly affecting the pathogen by an oxidative reaction, in the biosynthesis of phytoalexins, and in activating plant defence genes, as well as in the induction of acquired disease resistance. Evidence for a direct role of hydrogen peroxide in plant defence was provided by Wu et al. (1995) when they expressed the gene encoding glucose oxidase from *Aspergillus niger,* that catalyses the oxidation of glucose-yielding gluconic acid and hydrogen peroxide, in transgenic potato plants. An increased level of hydrogen peroxide was observed, resulting in a simultaneously increased resistance to infection in *Erwinia carotovora* ssp. *carotovora*.

Conclusions

Molecular approaches to the achievement of resistance to various pathogens frequently end up with the demonstration of increased protection in the laboratory or at best in the greenhouse, *i.e.* under artificial inoculation and growing conditions. The reasons for the products not entering the field may be numerous, including a missing link to breeders, lack of funding and also technical as well as experimental difficulties. Laboratory scientists may be short of expertise with the pathogen itself, while field experts on the disease may not consider all the aspects of working with transgenic organisms. In addition, it is often difficult to quantitate in a reproducible manner the degree of resistance in

the field due to the complex interaction between variations in the expression of the foreign gene and the permanently evolving pathogen, all within a changing ecology. It should therefore be stressed that similarly to the interaction between host and pathogen a permanent information flow and cooperation between laboratory and field scientists is of basic importance so that original concepts for novel integrated disease and pest management could be elaborated.

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Book reviews

CHARLES R. HENDERSON: Applications of Linear Models in Animal Breeding. University of Guelph, 3rd ed., 1994. 423. pp., ISBN: 0-88955-030-1

The great American scientist: Charles Roy Henderson (1911–1989) was born in Iowa, some 15 years after another great man in the field of animal breeding Jay L. Lush, who was also born in the same county. Henderson never forgot his farm background as he established a near perfect academic record at Iowa State College. He received an M.Sc. in animal nutrition and a Ph.D. degree in animal breeding at Iowa State University with Lush and Lanoy Hazel. He joined the faculty of Cornell University where he established methods for genetic evaluation and for the estimation of genetic variances that are an enduring legacy. After his retirement he continued his increasingly active professional career at many institutions around the world influencing animal breeding strategies.

His interest in deriving selection criteria from badly unbalanced data in the presence of a host of confusing environmental effects began with his Ph.D. thesis problem at Iowa State University. (In experimental design, the term 'unbalanced' refers to an experiment or set of data in which all treatments or treatment combinations are not equally represented. The most common cause of unbalanced experiments is unequal mortality among entries in a test.) It was concerned with the evaluation of single crosses between inbred lines of Poland China swine. The data represented measurements on the progeny of lines that differed in the levels of inbreeding of the sows, in the farrowing season and in the ages of the sows.

By 1947, the selection index had been developed as a method for selection among individuals with varying amounts of information. This method assumes that the records are expressed as deviations from the true means and assumes that the distribution is multivariate normal. However, the selection index theory provided no guidance with respect to how the means should be estimated in badly unbalanced data with a host of confusing fixed effects. By that time, quite a lot was known about the unbiased estimation of fixed effects in a fixed model using a least squares technique. In his thesis he tried to combine the method of least squares and the selection index to solve the problem of selection in unbalanced mixed models. In 1948 he began a long career in animal breeding at Cornell University and continued the research begun at Iowa State University on the genetic analysis of unbalanced data. At Cornell, he derived mixed model equations for Best Linear Unbiased Estimation (BLUE) and Best Linear Unbiased Prediction (BLUP), which have now become standard for the comparison of all competing evaluation methods in animal breeding. The BLUP value has been widely used for several decades to improve animal production. The use of BLUP values has proved to be successful in almost every field of animal production.

The need was felt to collect some of Henderson's publications on the prediction of breeding values and the estimation of variance components in a single volume in a systematic order and with a consistent notation. To fulfil this need, the book entitled *'Applications of Linear Models in Animal Breeding'* was compiled, which will be useful for teaching advanced courses in animal breeding and also as a reference work for researchers in this field. The book deals exclusively with the analysis of data arising from experiments or sampling schemes for which a linear model is assumed to be a suitable approximation.

The book is divided into 33 chapters. It gives a detailed review of different linear models used previously or currently in animal breeding. From the simplest regression models he presents and discusses the possibilities of linear unbiased estimations (BLUE), the prediction of random variables (BLUP, mixed models), biased estimation and prediction (BLBE, BLBP), the quadratic estimation of variances (Henderson's methods 1, 2, 3), Multivariate Invariant Quadratic Unbiased Estimation (MIVQUE) of variances and covariances, the effects of selection, the one-

way random model, the two- and three-way fixed models, the Animal Model, covariance models, the Sire Model, the genetic model, maternal effects, the three-way mixed models, the Restricted Maximum Likelihood Estimator (REML), the Simple and Multiple Regression Models, sire and cow evaluation, to name just a few. He did not attempt to give a comprehensive review of the literature of linear estimation and prediction or of the estimation of variances and covariances. Rather, he emphasized methods that seemed useful in animal breeding with a number of references to original works and to publications that present more comprehensive literature reviews. Most of the methods presented in this book are illustrated with numerical examples using small data sets for easier verification.

In spite of the clear-cut text that avoids the slightest superfluity (closely resembling a maths textbook), it is not easy reading, naturally enough. A thorough understanding of matrix techniques is a prerequisite to fully comprehending the models the book deals with. Some of the material in this book is taught to postgraduate students at different American universities. The American approach requires a profound knowledge of statistics. Since the models applied in the American animal breeding industry are different to those used in Hungary, it is advisable to be familiar with these techniques before undertaking a study trip to the US.

B. BARANYAI and Á. JÁNOSA

A. ALTMAN (ed.): Agricultural Biotechnology. 770 pp. Marcel Dekker Inc. New York, 1998, ISBN: 0-8247-9439-7

Conventional agricultural techniques, such as classical plant and animal breeding, have resulted in fantastic achievements during the last century. With the human population expected to reach 10 billion by the year 2050, the challenges facing agriculture and food research are enormous. Therefore, new methodologies are clearly required to intensify the development of agriculture throughout the world. Agricultural biotechnologies have seen dramatic developments during the last decade and should be able to overcome the remaining difficulties and expand the limits of conventional procedures. Only in the last two decades has it been possible to operate directly on the heart of biological matter, transcending the reproductive barrier between species and creating entirely new life forms. We can now make new varieties using genetic engineering, which are already being sold in some parts of the world.

The advent of the recombinant DNA technology heralded a second more radical Green Revolution of plants, animals and microorganisms engineered to defeat disease, pests and harsh conditions or to yield more and better produce with reduced inputs.

The book tries to give a complete review on the current state of modern agricultural biotechnology including the four major areas, i.e. microbial, plant, animal and marine biotechnologies. It contains 36 chapters written by 74 well-known contributors on 770 pages in 6 parts.

The **first part** (Plant Biotechnology) deals with the most commercially efficient types of micropropagation, including pathogen elimination and *in vitro* conservation. The asexual genetic methods include the manipulation of somatic and gametophytic cells and cell fusion. Finally, the genetic engineering of plants is discussed both for conventional breeding purposes and for industrial applications, including secondary metabolites and alien compounds.

The main subjects of **Part Two** (Microbial Agro-Biotechnology) are the biocontrol of bacteria and phytopathogenic fungi, the evaluation of insect- and herbicide-resistant transgenic plants and the biotechnological approaches to microbial fertilizers, phytostimulation, lignin degradation and the bioremediation of organic pollutants. Both animal and plant breeders face two very different challenges. One is to further increase productivity and the other is to provide varieties adapted to the different conditions in various parts of the world.

The **third part** (Animal Biotechnology) first deals with molecular breeding and marker-assisted selection, a topic which is

regrettably missing in the Part on Plant Biotechnology. Further chapters in this Part discuss the biotechnology of embryo manipulation, gender preselection and transgenic animals, applicable partly in conventional animal husbandry and partly in industrial production (e.g. novel proteins in milk).

Part IV (Marine Biotechnology) covers the subject of transgenic fish technology, including the production of growth hormone-transgenic salmon, and current approaches to the use of marine algae as a tool for the bioremediation of marine ecosystems. Finally, the valuable products of microalgal biotechnology are presented.

The most interesting aspects (legal and public) of Agricultural Biotechnology are discussed in **Part V.** These include the problems of laws, patents or variety certificates, intellectual property rights, bioethics, benefits and risks, scientific responsibility, safety aspects, environmental and social issues, etc.

The last part (**Part VI**) is an epilogue written by the distinguished scientists N. L. First, J. Schell, I. K. Vasil, D. J. Grimes and A. Sasson, on prospects and limitations, environmental interactions, and the relevance of this topic in developing countries.

In conclusion the handbook provides synthesized, up-to-date information on the practical application of molecular biology and gene technology methods in cultivated plants, livestock, fish, marine invertebrates, macro- and microalgae, all written by experts in their fields.

One of the weaknesses of the book is that the editors gave too much freedom to the contributors with regard to the contents and style of their chapters. The other is the insufficiency of illustrations in some chapters. In spite of these deficiencies the book will be very useful as a reference book. At the end of each chapter a great deal of literature is listed, the total number of citations being in the neighbourhood of four thousand. The book can be recommended not only for animal, plant, microbial and marine biotechnologists and experts, but also for teachers and students, and for the administrative staff of international organizations and state authorities.

L. HESZKY

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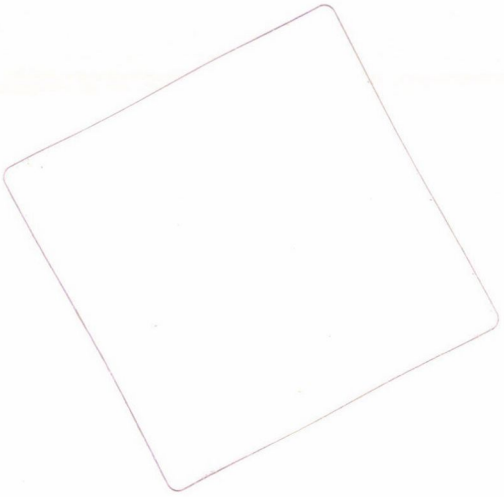

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CONTENTS

ORIGINAL PAPERS

Acta Agronomica Hungarica, 46(3), pp. 201 –207 (1998)

A SIMPLE AND EFFICIENT METHOD FOR THE TRANSFORMATION OF EGGPLANT (*SOLANUM MELONGENA* L.)

A. SZÁSZ, D. SZILASSY, K. SALÁNKI, M. FÁRI and E. BALÁZS

AGRICULTURAL BIOTECHNOLOGY CENTER, H-2101 GÖDÖLLŐ, P.O. BOX 411, HUNGARY

(Received: 13 May, 1998; accepted: 24 July, 1998)

A novel, time- and labour-saving protocol for the genetic transformation and regeneration of eggplant (*Solanum melongena* L.) is described. In this method, co-cultivation with *Agrobacterium*, callus regeneration and organogenesis are carried out in one step via the decapitation of rooted hypocotyls. Plantlets expressing the *NptII* gene as a selectable marker and the cucumber mosaic cucumovirus coat protein transgene were regenerated on TMR medium containing 100 mg/l kanamycin. The structural integrity and expression of the introduced viral gene were investigated and verified by PCR and Northern hybridization, respectively.

Key words: plant transformation; eggplant; cucumber mosaic cucumovirus (CMV)

Introduction

Eggplant is an important vegetable grown world-wide, especially in Asia and in the Mediterranean region of Europe. Among its diseases, the viral pathogen cucumber mosaic cucumovirus (CMV) is of great significance (Tanne and Zimmerman-Gries, 1980; Ding et al., 1989). The introduction of virus resistance genes from wild relatives via sexual crosses (Daunay et al., 1991) or somatic hybridization led to limited success (Gleddie et al., 1986; Guri and Sink, 1988a, 1988b; Sihachakr et al., 1988, 1989; Daunay et al., 1993). Therefore, genetic transformation appears to be a more effective strategy for providing eggplant cultivars with new resistance factors.

Stable *Agrobacterium*-mediated transformation has been achieved using leaf and cotyledon explants from *in vitro* plantlets (Guri and Sink, 1988c; Filippone and Lurquin, 1989; Rotino and Gleddie, 1990; Rotino et al., 1992; Fári et al., 1995a). In these experiments, the production of transgenic plants typically involved the following steps: co-cultivation of the explants with *Agrobacterium tumefaciens* for two days (following pre-culture in some cases), callus regeneration (2–3 weeks) and initiation of shoot regeneration (or embryogenesis; 2–3 weeks) initiation of root regeneration (2–3 weeks). While the numerous subculturings in these protocols involve the addition of exogenous growth regulators, the present paper describes a simple and efficient method which does not involve such steps. By the application of the ´Seedling Decapitation Method´ (Fári et al., 1992, 1995b), strong shoot proliferation was observed and hence a great transformation efficiency was achieved using the coat protein (CP) gene of CMV Trk7.

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Materials and methods

Plant material and culture media

Seeds of *Solanum melongena* L. cv. 'Kecskeméti lila' were surface-sterilized and placed into 425 ml 'Plant Box' plastic containers (Kontaplant Co., Ltd., Szentes, Hungary) containing 70 ml of TM medium (see below). Germination was performed at 25±1 °C under a 16/8 h photoperiod at a photon fluency rate of 35 μmol/m^2/s from F-29 fluorescent tubes (Tungsram, Hungary).

MSB_5 medium was composed of MS micro- and macroelements (Murashige and Skoog, 1962) and B_5 vitamins (Gamborg et al., 1968). Hormone-free MSB_5 medium with 20 g/l saccharose (TM) was used for the germination of seeds. This medium was completed with 0.05 mg/l BAP + 0.05 mg/l IAA (TMG) for shoot regeneration or with 0.5 mg/l IAA (TMR) for rooting.

Construction of transformation vector

The cloning and sequencing of the cDNA of CMV strain Trk7 have previously been reported (Salánki et al., 1994). The 1205 bp Xba I fragment, carrying the entire CP gene and the 3' untranslated region, was subcloned into the multiple cloning site of the plant expression vector pGA482 35S:NOS (Kollár et al., 1993) (Fig. 1). This plasmid contained the cauliflower mosaic virus 35S promoter upstream and the nopaline synthase (NOS) polyadenylation signal downstream of the multiple cloning site. The selectable marker gene neomycin phosphotransferase II (*NptII*) driven by nopaline synthase promoter was also carried within the T-DNA border sequences of the vector. This plasmid construct (pGACCP) was mobilized from *E. coli* DH5α to *Agrobacterium tumefaciens* LBA4404 (Hoekema et al., 1983) by a triparental mating procedure using the helper plasmid pRK2013 (Ditta et al., 1980).

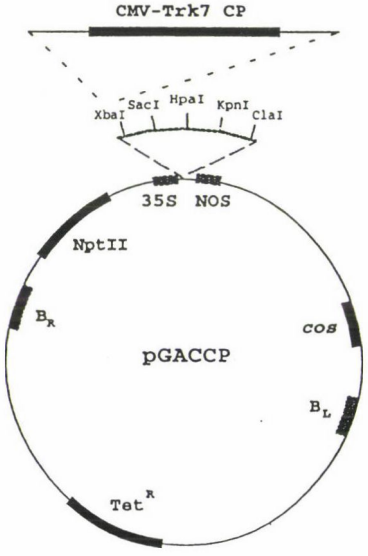

Fig. 1. Structure of the engineered *Agrobacterium* plasmid used for the transformation of eggplant. The 1205 bp *XbaI* fragment carrying the entire coat protein gene of the cDNA of CMV-Trk7 was cloned into the *XbaI* site of the *Agrobacterium*-derived broad host range vector pGA482 35S-NOS

Plant transformation and regeneration

Two hundred 14–21-day-old aseptic seedlings were decapitated slightly below the cotyledonary node. The inoculation was carried out simply by dipping the blade in a fresh overnight *Agrobacterium* culture prior to use. A small longitudinal cut was also made on the decapitated hypocotyls to prevent the drops from running off. After two weeks of cultivation the regenerated shoot bunches were cut and transferred to a selective TMG medium supplemented with 500 mg/l cefotaxime to kill the bacteria and 100 mg/l kanamycin as a selection agent. The green shoots obtained were transferred to TMR medium with the same cefotaxime and kanamycin concentration. Plants able to root on this selective medium were put into 500 ml jars on a sterile perlite:soil (1:1) mixture wetted with sterile distilled water. The jars were covered with polyvinyl chloride foil, which was removed gradually, helping to harden the plants.

Analysis of transformant plant lines

Total DNA was extracted from young leaf tissue according to Dellaporta et al. (1983). 500 ng DNA extract was used as template for the polymerase chain reaction (PCR). The following primers corresponding to the 35S promoter and the 3' end of the transgene, respectively, were applied :

5'-CCACTATCCTTCGCAAGACCC-3'
5'-GGATCCTGGTCTCCTTATGGAG-3'

Electrophoresis of the PCR products was performed according to Sambrook et al. (1989).

Total RNA was extracted from young leaf tissue by the LiCl method as described by Tumer et al. (1986). Samples containing 20 μg of RNA were electrophoresed in formaldehyde containing 1% agarose gel.

The blotting of the PCR products or RNA onto 'Hybond N' nylon membrane (Amersham) and the hybridization were carried out as described by Sambrook et al. (1989). Radio-labelled probes were made by random priming using a 'T7 Quick Prime Kit' (Pharmacia Biotech) from pCCP plasmid as template. This plasmid contained the 1205 bp Xba I fragment from the cDNA clone of Trk7-CMV RNA3, which included the entire CP gene, cloned into Bluescript SK+ vector.

Results

Plant regeneration and transformation

The engineered plasmid vector construct (Fig. 1) was mobilized into *Agrobacterium tumefaciens* LBA4404. Adventitious buds developed on the cut surface of their hypocotyls on 95% of the decapitated, *Agrobacterium*-infected seedlings (Fig. 2). The average number of shoot buds in the infected plants was similar to that in the non-infected control ones. Therefore, *Agrobacterium* infection did not seem to reduce the regeneration ability of these explants. Two weeks after decapitation, newly formed shoot bunches were transferred onto selective TMG medium. These small shoots started to grow, but most of them turned yellow and died after two weeks. There were nine independent shoot bunches which produced at least one shoot able to grow on this medium. These fifteen shoots were cut and transferred onto selective rooting medium (TMR), where, with the exception of one, they were able to form roots (Table 1). These shoots were considered as independent lines and were propagated *in vitro*. After transfer to the greenhouse they appeared phenotypically normal and set seeds, although a very limited amount of seeds was obtained, similarly to non-transgenic

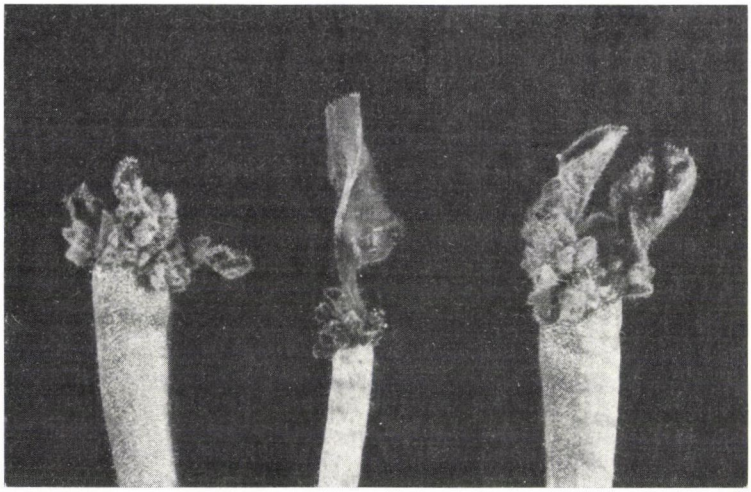

Fig. 2. Organogenesis on eggplant hypocotyls two weeks after decapitation

plants. Seeds of normal size were produced by lines 1-1, 6-2, 6-3, 6-4 and 24-3. Progeny seedlings of these lines developed well on medium containing 100 mg/l kanamycin at an average ratio of 83%, while the non-transgenic control plants did not develop roots and died (data not shown).

Analysis of transformant plant lines

The results obtained in the genetic analysis of putative transformant R_1 eggplant lines are given in Table 1. We focused on the CMV CP gene rather than on the *npt II* marker gene, so PCR was carried out with primer designed for the 35S promoter and viral 3' specific sequence to show the presence of the CMV CP gene in the kanamycin-resistant seedlings. The result of the PCR reactions for five independent lines is shown in Figure 3A. The identity of the PCR products was confirmed by Southern hybridization using a probe corresponding to the CMV CP gene (Fig. 3B). Transgene expression was detected by Northern hybridization in these primary transformants (Fig. 4). Despite the presence of the transgene mRNA, we were not able to detect the translation product by Western blot analysis (data not shown).

Table 1
The result of shoot regeneration and transformation*

Shoot bunches	1	2	3		4	5	6				24			98
Eggplant lines	1-1	2	3-1	3-2	4	5	6-1	6-2	6-3	6-4	24-1	24-2	24-3	98
PCR	+	+	+	–	+	+	+	+	+	+	+	+	+	–

*There were eight shoot bunches originating from independent decapitated seedlings which consisted of shoot buds able to form roots on TMR medium containing kanamycin. Twelve out of fourteen eggplant lines derived from these individual shoot buds were PCR positive.

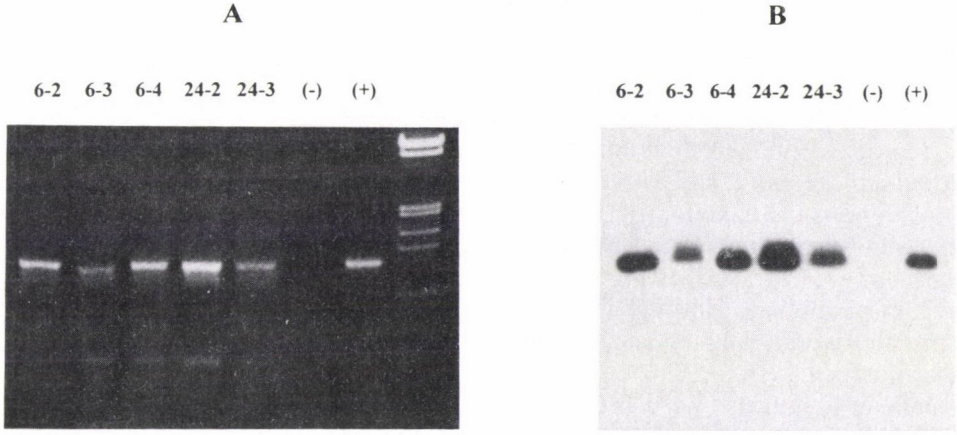

Fig. 3. The result of PCR analysis. Lanes 6-2, 6-3, 6-4, 24-2, 24-3 and (-) represent the corresponding transgenic lines and non-transgenic eggplant, respectively. The lane designated (+) is a PCR control in which pGACCP was used as template (A). The origin of the products was confirmed by Southern hybridization using a CMV CP specific probe (B)

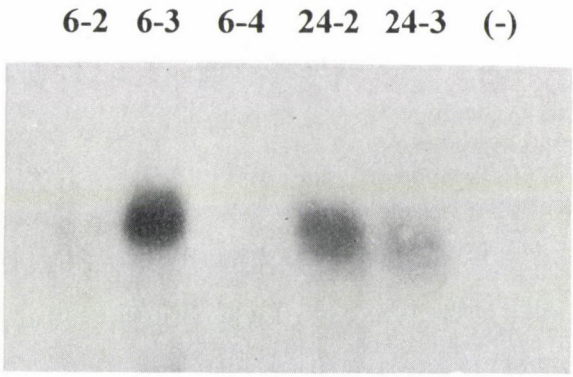

Fig. 4. Northern blot analysis of CMV CP transcripts in transgenic eggplants. Lanes 6-2, 6-3, 6-4, 24-2 and 24-3 are samples from the corresponding transgenic lines, lane (-) contains total RNA extract from non-transgenic eggplant

Discussion

In the present work, a labour- and time-saving organogenetic method was successfully employed in the transformation process of eggplant. While previous reports on eggplant transformation involved subculturing steps for several weeks (Guri and Sink, 1988c; Filippone and Lurquin, 1989; Rotino and Gleddie, 1990; Rotino et al., 1992; Fári et al., 1995a), here organogenesis and co-cultivation with the disarmed *Agrobacterium* vector was performed at the same time. Moreover, in contrast to earlier reports, no exogenously added growth regulators were required for shoot regeneration. This operation, called

the 'Shoot Decapitation Method' (SDM), has already been described in tomato (*Lycopersicon esculentum* Mill.), eggplant (*Solanum melongena* L.) and in a species of *Capsicum* (*Capsicum pendulum* L.) (Fári et al., 1992, 1995b). The ability to initiate regeneration on the cut surface of hypocotyls has also been observed in other members of the *Solanaceae* family: in *Solanum capsicastrum*, *S. muricatum* and *S. sodomeum* (Fári, not published).

The transformation of a cultivated eggplant variety was carried out with an economically important gene derived from a cDNA clone of the CMV isolate Trk7. The integration of the CMV CP gene was shown by PCR in fourteen chosen plant lines and their expression by Northern hybridization in five. The decreased ability of the plants to produce normal seeds could not be attributed to the transformation and *in vitro* regeneration process, as it was also observed for untreated eggplants in the greenhouse (not shown). A probable explanation is that this variety is not well adapted to greenhouse conditions.

The present study demonstrates that decapitated, rooted hypocotyls of *Solanum melongena* can be transformed by a simple *Agrobacterium* co-cultivation method. This technique is applicable in practice and plants expressing CMV coat protein gene could provide valuable virus-resistant stock material for conventional breeding.

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IDENTIFICATION OF CHROMOSOMAL TRANSLOCATIONS IN COMMON WHEAT, DERIVATIVE OF *TRITICUM TIMOPHEEVII*

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By analysing the pollen mother cells at the first metaphases of meiosis in F_1 hybrids produced in crosses between the common wheat line 146–155–T (derivative of *Triticum timopheevii*) and a set of 21 monosomic lines of cv. Chinese Spring, chromosomal interchanges were identified. On the basis of the frequency of trivalent configurations it was found that two reciprocal translocations, involving chromosomes 3A and 4A on the one hand, and chromosomes 6B and 7D on the other, differentiated the line 146–155–T from cv. Chinese Spring. It seems probable that in the F_1 hybrids (Chinese Spring x 146–155–T) genes causing a decrease in pairing are found on chromosomes 1A, 3A, 2B and 6D, and genes enhancing pairing on chromosomes 6B, 1D, 4D and 5D. The participation of the 6B chromosome in the interspecific translocations was confirmed by C-banding analysis of mitotic chromosomes and by electrophoretical analysis of storage proteins in wheat line 146–155–T and its parent species.

Key words: meiotic behaviour, chromosomal interchanges, monosomic analysis, C-banding, wide hybridization, *Triticum aestivum*, *Triticum timopheevii*, electrophoresis of gliadin

Introduction

Many agronomically important traits, including resistance to diseases and pests, have been transferred to common wheat, *Triticum aestivum* L., from relative species of the subtribe *Triticinae* using wide hybridization (Gale and Miller, 1987; Friebe et al., 1996). The widening of genetic variation in common wheat germplasm, using the transfer of alien genetic material from wild relatives, results in extensive exchanges of chromosomes or chromosome segments, and reciprocal translocation formations.

Hexaploid wheat cultivars (*Triticum aestivum*, 2n=6x=42) are often differentiated by one or more reciprocal translocations, and it is possible to identify the number of these chromosomal interchanges relative to cultivar Chinese Spring after crossing the wheat genotype under study and the monosomic set of Chinese Spring lines (Riley et al., 1967; Baier et al., 1974; Sutka, 1978). The "critical" chromosomes involved in translocation are frequently identified after crosses and the cytological analysis of meiosis in F_1 hybrids (Law and Worland, 1972).

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The grain storage proteins can be used to reveal translocations and substitutions (Gupta and Shepherd, 1992). The genes which code for the storage proteins occur at nine very well-known complex loci on homoeologous group 1 and group 6 chromosomes. Gliadin subunits are coded by genes located at the Gli-1 and Gli-2 loci on the short arms of the group 1 and group 6 chromosomes, respectively, and the high-molecular-weight (HMW) glutenin subunits are coded by genes located at the Glu-1 loci on the long arms of the group 1 chromosomes of hexaploid wheat (Payne et al., 1980).

The present paper deals with the identification of chromosomal translocations revealed at meiosis and at mitosis on C-banded chromosomes and the electrophoretical analysis of storage proteins in the introgressive common wheat line 146–155–T, a derivative of the tetraploid wheat species *Triticum timopheevii* Zhuk.

Materials and methods

Plants of the common wheat mutant 146–155, induced in the cv. Norröna after mutagenic treatments with N-methyl-N-nitrosourea, were used as female parent for crossing with the tetraploid wheat species *Triticum timopheevii* Zhuk. (2n=28, genome formula AAGG), which is used in wheat breeding as a donor of disease resistance. Plants of the pentaploid F_1 hybrid were backcrossed to the female parent to restore fertility. After screening and estimation in artificial inoculation conditions the line 146–155–T, with resistance to leaf rust (*Puccinia recondita* f. sp. *tritici*) and powdery mildew (*Erysiphe graminis* f. sp. *tritici*), was selected from backcrossed advanced generations (Peusha et al., 1995; 1996).

Chromosome pairing in pollen mother cells (PMCs) at the 1st meiotic metaphase (MI) was analysed in F_1 hybrid progeny produced in crosses between each of the 21 monosomic lines of cv. Chinese Spring and the introgressive line 146–155–T. The somatic chromosome numbers of the Chinese Spring monosomic plants and the F_1 monosomic hybrids were determined using the Feulgen method. For the cytological examinations, young anthers, containing PMCs at the first metaphase of meiosis, were fixed in 3 : 1 absolute alcohol-glacial acetic acid and prepared using the acetocarmine squash method. Every PMC was scored for the presence of univalents, bivalents and multivalents, and where multivalent associations occurred, the number of chromosomes involved and their configurations were recorded. The statistical analysis and comparisons between the meiotic behaviour of the different hybrids were made using a Student's t-test.

For the C-banding procedures, seeds of the mutant wheat 146–155, the hybrid line 146–155–T and *T. timopheevii* were germinated in Petri dishes on moist filter paper for 48h at 25°C. Root tips (1–2 cm long) were excised and transferred to ice water for 26h at 0°C. Pretreated root tips were then fixed in ethanol-glacial acetic acid (3:1) for 3 days, stained with 1% acetocarmine solution for 1–2h at room temperature and squashed in 45% acetic acid. The cover slip was removed by freezing in liquid nitrogen. The slides were transferred to 70% ethanol for a few minutes and then air dried. The preparations were then incubated in 0.2M HCl at 60°C for 2 min and at 37°C for 10 min, washed in distilled water, incubated in saturated barium hydroxide solution at 35°C for 20 min, rinsed in distilled water, and incubated in 2xSSC at 60°C for 1h. The chromosomes were stained with 1% Giemsa in phosphate buffer for up to 2h.

Electrophoresis of gliadin was performed by the method of Metakovsky and Novoselskaya (1991). Gliadins were extracted from single grains using 70% ethanol. Electrophoresis was performed on 10% polyacrylamide gels at 500V constant current and stained with Coomassie Blue R-250 (Bushuk and Zillman, 1978).

Results and discussion

The cytological analysis of meiosis in the monosomic F_1 hybrids from the cross Chinese Spring × 146–155–T has revealed less regular chromosome pairing at MI compared with both the parents and disomic F_1 hybrid. Significant differences were observed in the range and mean of bivalents and univalents in the monosomic crosses. Associations between the chromosomes in the monosomic hybrids were rather weak, resulting in premature disjoining of the bivalents and an increase in the number of additional univalents. The mean number of bivalents was lower and that of univalents higher in monosomic crosses with the 1A, 3A, 2B and 6D chromosomes. The mean number of bivalents was greater and that of univalents lower in monosomic hybrids 6B, 1D, 4D and 5D; in these crosses the mean number of chiasmata (35.8; 36.0; 36.2 and 36.0, respectively) was also higher (Table 1). It was found that some chromosomes of the introgressive line 146–155–T affected pairing at the MI of meiosis: chromosomes 1A, 3A, 2B and 6D decreased pairing, whereas chromosomes 6B, 1D, 4D and 5D increased it.

Table 1

Chromosome pairing at MI in monosomic F_1 hybrids from crosses between Chinese Spring monosomics and introgressive line 146–155–T

Monosomic line	No of PMCs	Mean number per cell[+]						%*	No. of PMCs**		
		Bivalents			Univalents	Chiasmata	Multivalents				
		Ring	Rod	Total					I^{III}	I^{IV}	I^{V}
1A	87	14.2	4.3	18.5 (15–20)	3.7 (1–9)	32.8 (26–38)	0.04 (0–1)		3	1	–
2A	37	16.3	3.0	19.3 (17–20)	2.3 (1–7)	35.6 (27–40)	0.02 (0–1)		–	1	–
3A	251	14.0	4.6	18.6 (15–20)	3.6 (1–11)	32.7 (22–40)	0.01 (0–1)	0.4	3	1	–
4A	84	13.7	4.7	18.4 (15–20)	3.2 (1–11)	32.8 (25–40)	0.20 (0–1)	3.6	10	9	–
5A	130	15.9	2.9	18.8 (14–20)	2.9 (1–7)	33.8 (26–40)	0.06 (0–1)		4	4	–
6A	138	14.8	3.7	18.5 (15–20)	3.8 (1–11)	33.3 (25–36)	0.02 (0–1)		1	2	–
7A	62	15.9	3.1	19.0 (17–20)	2.7 (1–7)	34.3 (30–39)	0.03 (0–1)		1	1	–
1B	132	14.5	4.2	18.7 (16–20)	3.3 (1–9)	33.3 (23–40)	0.04 (0–1)		2	4	–
2B	70	14.1	3.9	18.0 (15–20)	3.9 (1–11)	32.8 (26–39)	0.28 (0–1)		9	11	–
3B	68	14.4	4.3	18.7 (16–20)	3.4 (1–9)	33.1 (25–37)	0.01 (0–1)		1	–	–
4B	120	15.9	3.1	19.0 (16–20)	2.7 (1–7)	35.1 (28–40)	0.05 (0–1)		1	6	–
5B	64	15.3	3.5	18.8 (16–20)	3.2 (1–7)	34.1 (28–40)	0.01 (0–1)		–	–	1
6B	54	16.2	3.2	19.4 (17–20)	1.6 (1–5)	35.8 (28–40)	0.12 (0–1)	7.4	6	1	–
7B	45	15.9	3.4	19.3 (18–20)	2.2 (1–5)	35.3 (30–39)	0.04 (0–1)		1	1	–
1D	181	16.6	2.7	19.3 (16–20)	2.2 (1–5)	36.0 (30–40)	0.01 (0–1)		–	2	–
2D	147	16.6	2.6	19.2 (16–20)	2.5 (1–7)	35.8 (30–40)	0.01 (0–1)		1	–	1
3D	95	16.8	2.2	19.0 (14–20)	2.7 (1–7)	35.8 (24–40)	0.03 (0–1)		1	2	–
4D	111	16.7	2.6	19.3 (18–20)	2.1 (1–5)	36.2 (31–39)	0.03 (0–1)		2	2	–
5D	139	16.6	2.7	19.3 (16–20)	2.2 (1–5)	36.0 (27–40)	0.03 (0–1)		–	5	–
6D	150	14.5	4.0	18.5 (15–20)	3.9 (1–11)	33.1 (23–39)	0.02 (0–1)		1	1	1
7D	79	15.3	3.7	19.0 (17–20)	2.3 (1–7)	34.8 (28–38)	0.18 (0–1)	12.6	13	2	–
CS dis.× 146–155–T	200	17.5	2.8	20.3 (18–21)	1.4 (2–6)	37,4 (32–42)	0.01 (0–1)		–	2	–
CS	156	19.9	0.9	20.8 (20–21)	0.2 (0–2)	40,8 (38–42)	0		–	–	–
146–155–T	105	20.2	0.7	20.9 (20–21)	0.1 (0–2)	41.1 (38–42)	0		–	–	–

[+]Range is given in parenthesis; * % of PMCs with trivalents, without univalents; **with multivalents

Analysis of the chromosome configurations at MI showed that the mean value of rod bivalents per PMC in introgressive line 146–155–T was 0.7, and in cv. Chinese Spring 0.9, while in monosomic crosses these values were significantly higher, averaging 2.8 (2.2 to 4.7) rod bivalents per PMC.

The existence of structural differences between the chromosomes of Chinese Spring, on the one hand, and the chromosomes of introgressive line 146–155–T, on the other, is supported by the data of chromosome configurations and multivalent formations in monosomic F_1 hybrids (Fig. 1). It is known that hexaploid wheat cultivars are often differentiated by reciprocal translocations. Chromosomes involved in translocations are frequently identified after crossing and analysing the F_1 hybrids. A chromosomal interchange is present when part of a chromosome is exchanged with part of another one. The presence of reciprocal translocations between the chromosomes of different wheat genotypes leads to irregular pairing and multivalent formation at MI. Most frequently wheat cultivars have from one to three or four reciprocal translocations relative to Chinese Spring, a common wheat cultivar with a standard chromosome structure (Vega and Lacadena, 1982).

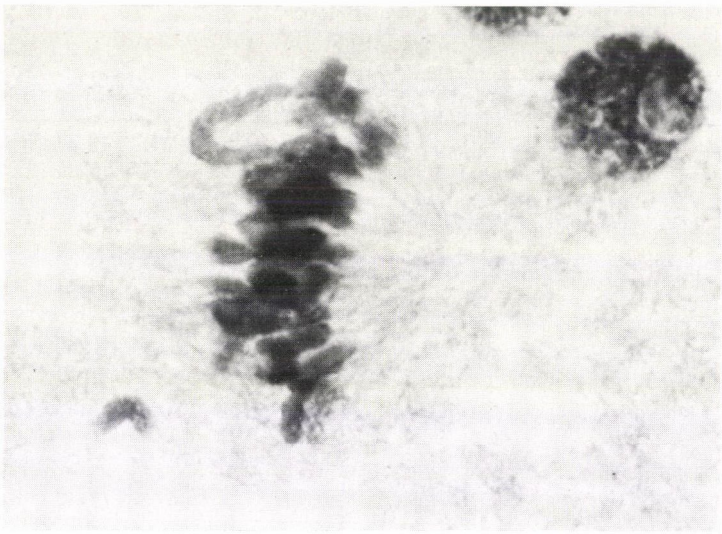


Fig. 1. First metaphase of meiosis in the F_1 hybrid between Chinese Spring-mono 7A and introgressive line 146–155–T showing one quadrivalent and one univalent ($18^{II} + 1^{IV} + 1^{I}$)

The present study demonstrates the occurrence of reciprocal translocation in the common wheat line 146–155–T. Among the 21 monosomic cross combinations tested, four carried chromosomal translocations, and in the other monosomic hybrids different frequencies of multivalent associations were observed. In some monosomic crosses PMCs with trivalent configurations were observed with or without the presence of univalents. In the monosomic hybrids, in which one of the chromosomes implicated in the translocation is absent,

trivalent configurations appear at MI (Law and Worland, 1972). The "critical" F_1 hybrids will have trivalents without univalents in a number of PMCs, while the "non-critical" hybrids have quadrivalent configurations. As is evident from Table 1, hybrids with monosomic lines 3A, 4A, 6B and 7D have trivalent configurations without univalents, and, consequently, these chromosomes are involved in translocations. On the basis of the frequencies of trivalent formation it can be assumed that introgressive line 146–155–T has the chromosomal interchanges 3A/4A and 6B/7D in relation to Chinese Spring. The low frequency of trivalent associations indicates that fairly small chromosome segments are involved in the translocations. The only exception is the interchange 6B/7D, which includes comparatively large segments of chromosomes 6B and 7D with 7.4 and 12.6 per cent, respectively, of trivalent associations.

The participation of the 6B chromosome in interspecific translocations was confirmed by means of the C-banding analysis of mitotic metaphase chromosomes in wheat line 146–155–T and the parent species and by electrophoresis of the gliadins. *T. timopheevii* and *T. aestivum* chromosomes were identified according to standard karyotype classification (Gill et al., 1991; Badaeva et al., 1994). A comparison of the 6B chromosome of the 146–155–T hybrid line with the 6B chromosome of the 146–155 wheat line and the 6G chromosome of *Triticum timopheevii* showed few differences in their banding patterns. Figures 2a, b and c show the C-banded karyotypes of 146–155, *T. timopheevii* and 146–155–T, respectively. Figure 3 demonstrates photographs of the 6B, 6G and 6B/6G translocated chromosomes. The 6G chromosome of *T. timopheevii* is one of the two nucleolus-organising satellited chromosomes. It shows clear interstitial heterochromatin in the nucleolus-organising part and a very small telomeric band like two dots or "eyes". The satellited 6B chromosome of *T. aestivum* also has strong interstitial heterochromatin, which is more extended than in chromosome 6G, but has no telomeric dots. Both the 6G chromosome of *T. timopheevii* and the 6B chromosome of wheat line 146–155 have very similar strong heterochromatic centromeric bands extending to both chromosome arms. In the hybrid line 146–155–T, the centromeric heterochromatin has less prominent bands. The interstitial heterochromatin in the nucleolus-organising part of the hybrid line 146–155–T looks like the interstitial band on chromosome 6G. The appearance of a small telomeric heterochromatic band in 146–155–T may indicate its belonging to the 6G chromosome of *T. timopheevii*. However, the centromeric part and the other chromosome arm seem to be unlike either 6B or 6G.

Based on C-banding analysis carried out earlier, it was assumed that line 146–155–T possessed a 6B(6G) chromosome substitution (Badaeva et al., 1995). The transfer of alien chromatin from *T. timopheevii* into the 146–155–T genome was suggested by fluorescent cytological analysis with genomic *in situ* DNA hybridization (GISH) (Enno et al., 1995). In this analysis, translocations of *T. timopheevii* chromatin into the 146–155–T genome were located on chromosome 6B (Järve et al., 1996). It is therefore assumed that the alien chromatin was transferred by genetic recombination through crossing over rather than by substitution of whole chromosomes of the wild species.

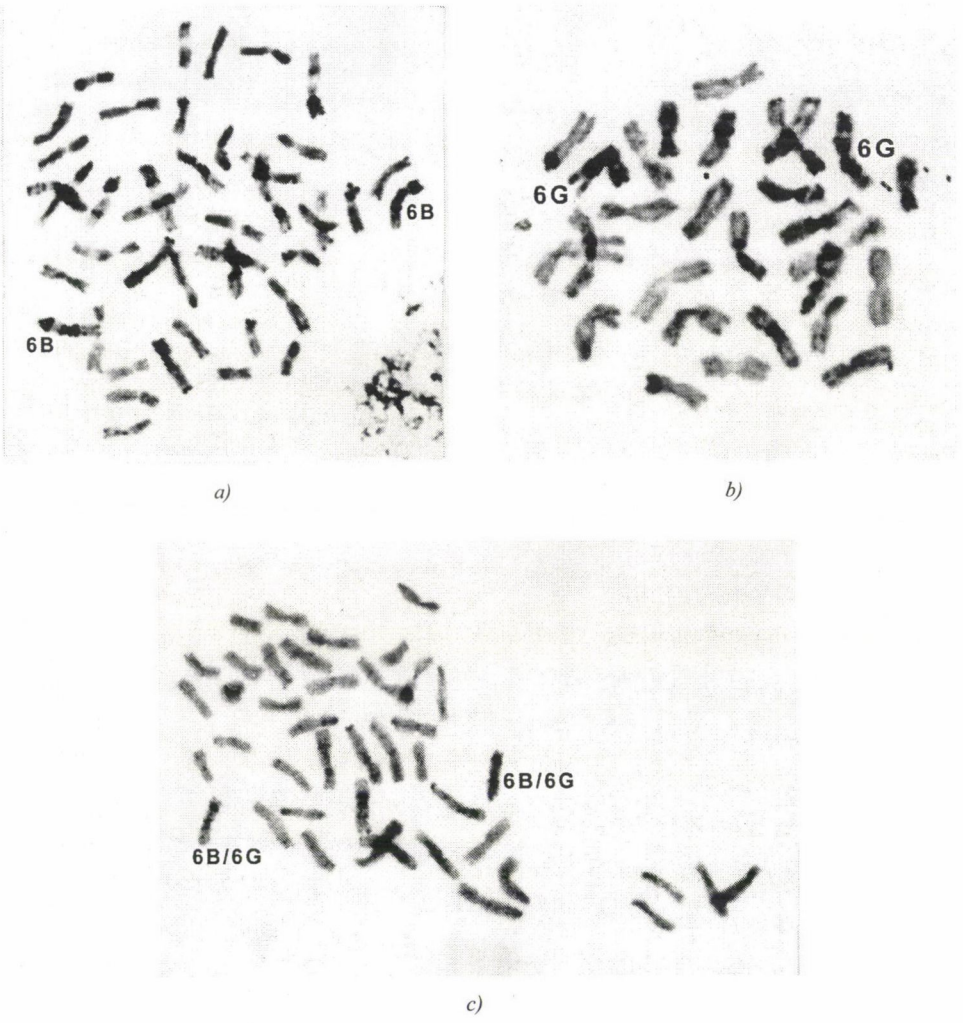
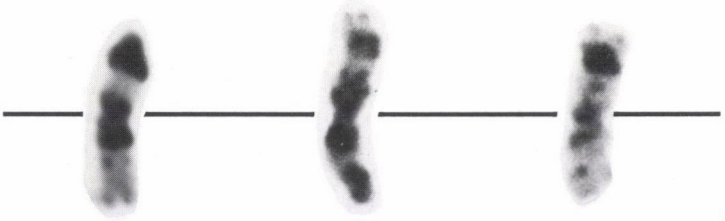


Fig. 2. C-banded mitotic metaphase chromosomes of the hexaploid wheat line 146–155 (a), the tetraploid wheat *Triticum timopheevii* (b) and the selected introgressive line 146–155–T (c)

The distribution of the gliadin components in the protein spectrum of 146–155, *T. timopheevii* and 146–155–T is presented in Figure 4. The bands in the hybrid line were complexes of the 146–155 and *T. timopheevii* bands. The gliadin spectrum of 146–155–T lacks the bands of 146–155 and has those of *T. timopheevii*. The synthesis of these proteins is carried out under the control of the 6B chromosome. Arrow-heads point to the controlled bands of 146–155 substituted by 6B. On the grounds of these experiments it was concluded that line 146–155–T possessed a 6B(6G) translocation.



6B 6G 6B/6G

Fig. 3. C-banding of chromosome 6B of wheat line 146–155, chromosome 6G of *T. timopheevii* and translocated chromosome 6B/6G of the introgressive line 146–155–T

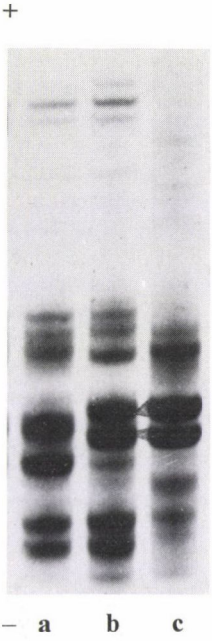


Fig. 4. Gliadin electrophoretic spectrum of 146–155, *T. timopheevii* and 146–155–T. Arrowheads indicate bands transmitted from *T. timopheevii* to the hybrid. a - 146–155, b - 146–155–T, c - *T. timopheevii*.

It can be concluded that the biochemical, cytological and monosomic analyses of introgressive wheat lines have an important influence on our understanding of wheat genetics and cytogenetics and will continue to play a role in the future, particularly in association with the development of new molecular-genetical marker techniques.

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COMPARISON OF ANTHER CULTURE CHARACTERISTICS AND SPONTANEOUS GENOME DOUBLING IN ANDROGENIC PLANTS USING MAIZE (*ZEA MAYS* L.) HYBRIDS WITH VARYING DH LINE PARENTAGE

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Studies were made on the haploid induction ability of hybrids originating from crosses between highly androgenic exotic DH lines and recalcitrant commercial inbreds. The results of anther induction after the first harvesting period suggest that all the hybrids studied in this experiment had a certain androgenic capacity; the highly androgenic DH lines are thus good sources for the introduction of haploid induction ability into non-responsive elite lines via crossing. Besides the early harvested embryos, secondary somatic embryos also provided a considerable number of plantlets. The transplantation of the plantlets into soil appeared to produce a physiological shock, resulting in a reduced number of adult plants. The spontaneous chromosome doubling rate (calculated on the basis of surviving regenerants) was very low in the genotypes examined, and only 2.4% fertile plants could be achieved from the cultured embryoids in the case of the best responding single-cross hybrid. These results indicate the necessity of an improvement in culture conditions in the plant regeneration phases and the elaboration of an efficient genome doubling technique.

Key words: anther culture, plant regeneration, spontaneous genome doubling rate, maize

Introduction

The potential of doubled haploids (DH) in maize breeding has long been recognized (Chase, 1969). The superiority of the DH breeding scheme lies in its unique form of gamete selection (Kasperbauer et al., 1980), which permits both dominant and recessive traits to be expressed. Furthermore, by doubling haploid genomes to obtain DH lines, additive genetic variance is doubled while dominance variance is eliminated (Choo and Kanneberg, 1978). The success of producing haploid plants in maize through anther culture makes it possible to generate inbred lines through chromosome doubling (Kuo et al., 1986) or to select for spontaneously doubled haploid plants from such cultures (Kovács et al., 1992). However, the successful application of anther culture techniques in maize breeding is largely dependent on the androgenic response of the genotypes and on the frequency of induced or spontaneous genome doubling in the regenerants. Although commercial hybrids were produced using the anther culture technology by the early eighties (Wu et al., 1983), maize remained a

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recalcitrant species with regard to *in vitro* androgenesis when compared to other cereals (e.g. barley, wheat and triticale). As most of the anther culture-responsive maize stocks have been found in non-commercial maize germplasm (Genovesi and Collins, 1982; Petolino and Jones, 1986; Pescitelli et al., 1989; Barnabás and Kovács, 1992; Antoine-Michard and Beckert, 1997; Orosz and Barnabás, 1997), Dieu and Beckert (1986) suggested that culturability should be transferred from the responsive, non-commercial, exotic germplasm (mainly of Chinese origin) into elite types. Furthermore, anther culture *per se* constitutes a selective process for increased androgenesis (Pickard and DeBuyser, 1977). The androgenic response in maize is a highly heritable trait (Cowen et al., 1992; Murigneux et al., 1994), so the production of highly responsive genotypes has become the main target of research world-wide in recent years. Using highly responsive genotypes, significant progress was achieved by intermating non- and highly responsive genotypes (Petolino et al., 1988).

During the last few years several highly androgenic genotypes have been produced at the Department of Cell Biology and Plant Physiology of the Agricultural Research Institute in Martonvásár, making it possible to introduce androgenic capacity into commercial inbreds. The aim of the present work was to study the anther culture response of a hybrid originating from two related DH lines as well as of two hybrids the parentage of which was 50% DH and 50% non-responsive lines derived by self-pollination.

Materials and methods

Plant material

In the present experiment the following maize hybrid genotypes were used as anther donor plants: A 632 × DH241, DH241 × DH 309 and (DH241 × DH 309) × 58, where 58 is an inbred from a synthetic population. A 632 (open pedigree line from Minnesota) and 58 (proprietary line of ARI HAS, Martonvásár) are non-responsive commercial inbred lines (Kovács et al., 1992; Barnabás et al., unpublished results). The anther response of the two commercial lines was studied without (Kovács et al., 1992) and with cold pretreatment (Barnabás et al., unpublished results) and they were found to be recalcitrant. The highly androgenic dihaploid line (DH 241) and the hybrid (DH241 × DH 309) were both produced in the experimental nursery of the Agricultural Research Institute of the Hungarian Academy of Sciences, Martonvásár, Hungary using the "direct chromosome doubling" method (Barnabás et al., 1991). These two genotypes are genetically closely related, since the DH lines were produced by the self-pollination of two anther culture-derived plants of the Chinese hybrid 592 × A2.

The anther donor plants were raised in climate chambers using the "Bk" programme elaborated by Barnabás and Rajki (1976) for the phytotronic cultivation of maize (Tischner et al., 1997). Tassels were collected prior to the emergence of the leaf sheath, when the microspores were judged to be at the mid-uninucleate stage of development.

Anther culture

After collection, the excised tassels were covered with aluminium foil and stored at 7 °C for 10 days as a cold pretreatment. After this 10-day period of cold pretreatment a few anthers from different parts of the tassel were removed, and were examined microscopically to verify the developmental stage. Parts of the tassels which contained mid- or late-uninucleate microspores were surface sterilized with 20% sodium hypochlorite for 20 minutes and then washed three times

with sterile distilled water. The anthers were isolated under aseptic conditions and inoculated onto the surface of F medium (modified YP medium, Genovesi and Collins, 1982) supplemented with 0.1 mg/l 2,3,5-TIBA, 5 g/l charcoal, 500 mg/l casein hydrolysate, 120 g/l sucrose and 2.5 g/l Gelrite at pH 5.8. The induction medium was autoclaved using the usual protocol. The cultures were incubated in the dark at 29°C for 28 days. After 28 days the number of responding anthers and the number of induced structures (embryo-like structures, ELS, or calli) were harvested and counted. After the embryoids were harvested the inoculated anthers were cultured for another three weeks to test if additional embryoids emerged from the responding anthers. After three weeks the newly emerged ELS were harvested and counted again and the capacity for secondary embryoid formation was determined in each case.

Plant regeneration

After the induction period the ELS and calli were removed from the anthers and the ELS were transferred directly to a regeneration medium. The harvested calli were used in other experiments to obtain haploid cell cultures. The regeneration medium used in this experiment consisted of MS macronutrients, MS microelements and RM vitamins supplemented with 1 mg/l kinetin, 0.5 mg/l NAA (naphthyl-acetic acid), 2 g/l sucrose and 7 g/l agar at pH 5.8 and autoclaved for 20 minutes at 121°C. The plant regeneration was carried out under 16 hours illumination (50 mMol/s/m^2 light intensity) at constant 26°C. The ELS and calli were cultured under these conditions in Petri dishes until some of the plantlets grew to 1–1.5 cm long, after which the green regenerants were transferred into glass containers for further growth using the same culture medium, but without hormones. In some cases 0.1 mg IBA was used for rooting. After two weeks, the plantlets were transplanted into peat pellets 5 cm in diameter (AS Jiffy Products Ltd.) and covered with plastic foil for around 5 days, to maintain high relative humidity. Finally the regenerants were transplanted to soil in 27.5 cm pots in growth chambers. Plants with normal plant behaviour were self-pollinated and they were grown till maturity according to the "Bk" maize plant growth programme. The fertility of the regenerants was evaluated on the basis of seed setting per plant.

The experimental data obtained were statistically analysed using the general method of ANOVA (SPSS for Windows, version 6.0) for induction and plant regeneration, while the significant differences in fertility were measured using the Mann-Whitney U-test (Goldstein, 1965).

Results

ELS and calli emerged from the cultured anthers within 3-4 weeks of culture initiation. Most often the responding anthers produced primary embryoids and very rarely multiple embryoids. In the case of the single-cross hybrid between the two androgenic DH lines about 50% of the responding anthers produced primary ELS, and when they were cultured for a period longer than 4 weeks, a considerable amount of secondary embryogenesis was observed (data not shown).

The results of anther induction (Table 1) after the first ELS harvesting period (4 weeks) suggest that all the hybrids studied in this experiment had a certain androgenic capacity, giving enough embryoids or calli for further studies. According to the data there were no significant differences in anther response between the different single-cross and three-way cross hybrids, even when one of the parents was completely recalcitrant. No significant difference was found in the frequency of haploid induction at the first harvest time,

although the single-cross hybrid between the two DH lines produced more haploid structures than the other hybrids. All the genotypes studied produced a significant number of haploid structures during the second 3 weeks of subculture, suggesting that the culture efficiency could be improved by further culturing after the 1 month culture period generally used. According to the data obtained, the total haploid induction frequency was high in all cases, though the three-way cross hybrid gave significantly less haploid induction than the single-cross hybrid of the two DH lines (Table 1). The main difference was found in anther productivity (the average number of primary structures induced per responding anther), where DH241 × DH309 gave a significantly higher number of primary embryoids (3.09) than the three-way cross hybrid (2.2), while the single cross between the line DH241 and the inbred A632 gave an intermediate result (2.5). Secondary (adventitious) embryo formation on the induction medium was observed only in the case of the single-cross hybrid between the two DH lines during the subculture period (data not shown).

Table 1
Anther response and haploid induction frequency of different maize hybrids in anther culture

Genotype	No. of anthers inoculated	Anther response (%)	Haploid induction at first harvest (%)	Haploid induction at second harvest (%)	Total haploid induction (%)
A632 × DH241	500	15.2 ns	27.2 ns	10.4 ns	37.6 ab
DH241 × DH309	1000	19.2 ns	40.8 ns	18.6 ns	59.4 a
(DH241 × DH309) × 58	500	13.8 ns	21.6 ns	9.2 ns	30.8 b

ns - non-significant; means within the same column followed by different letters are significant ($P< 0.05$)

The plant regeneration data (Table 2) showed that the plant regeneration capacity of the cultured ELS was still limited. The overall ratio of generation from ELS to plant is certainly lowered by the fact that a relatively high number of ELS do not develop normally and remain at a globular or polar embryo stage or simply produce calli. The embryoids which developed into plantlets showed different developmental pathways; some of them regenerated via organogenesis while others followed the normal embryogenic pathway. In some cases plant regeneration via secondary embryogenesis was observed, especially in the case of the three-way cross hybrid. On average there was no significant difference between the genotypes studied, but they had different sensitivities to transplantation into soil. The data presented in Table 2 represent the number of plantlets adapted to soil conditions. The plantlet regeneration frequency under aseptic conditions was generally higher (around 25%), but serious adaptational problems were observed independently of the genotype. The plantlets already

adapted to the soil showed phenotypically different characteristics. The haploid plants regenerated in this experiment all displayed a characteristic morphology (short, narrow leaves, reduced vigour, no pollen shed and in many cases feminization), while the spontaneously doubled haploids were generally more vigorous in appearance and grew more rapidly compared to the haploid plants. The spontaneous doubling rate was very low, and showed a strong significant genotypic difference, especially in the case of the three-way cross hybrid, where no fertile plants were obtained at all. The two single-cross hybrids gave an acceptable spontaneous doubling rate.

Table 2

Plant regeneration capacity of the ELS obtained from anther culture and the effect of genotype on the spontaneous genome doubling rate

Genotype	No. of embryoids cultured	Plant regeneration		No. of fertile plants	% of fertile plants	Spontaneous doubling rate
		No.	%			
A 632 × DH241	148	26	17.5 ns	3	11.5a	0.15a
DH 241 × DH 309	373	48	12.9 ns	9	18.4a	0.18a
(DH241 × DH309) × 58	84	6	14.9 ns	0	0.00b	0.00b

ns - non significant; means within the same column followed by different letters are significant ($P<0.05$)

The doubled haploid plants from the different hybrids exhibited similar morphology. Most of them produced abundant, viable pollen, but as regards the sexual organs, some of the plants (around 70%) showed sectorial fertility and tassel seed was very frequent, suggesting that the plants might be mixoploid or chimeric. The ears of these plants could, however, still be self-pollinated, as the silks emerged in time in all cases (Table 3).

Table 3

Relationship between the delay in silk emergence after the beginning of pollen shed and the average number of seed produced per ear on spontaneously doubled haploid plants

Genotype	Silk emergence delay (days)	No. of plants pollinated*	Average seed per ear
A 632 × DH241	1	0	0
	2	2	81
	3	1	78
DH241 × DH309	1	1	105
	2	6	89
	3	2	41

* One ear per plant was self-pollinated in each case.

Most of the doubled haploid plants produced from 4 to 145 seeds per ear after pollination, but in general the seed setting was far below the normal seed set of self-pollinated hybrids (400 seeds/ear on average). There was no significant difference between the two genotypes giving spontaneously doubled plants in the synchronity of pollen shed and silk emergence. As shown in Table 3, the silks emerged for pollination 1–3 days later than the first pollen was shed, and an average acceptable number of seeds was set.

Discussion

The experimental results underline the statement of other experts that the androgenic response can be transmitted into recalcitrant elite lines by crossing (Dieu and Beckert, 1986; Barloy et al., 1989). Both the DH lines of Chinese origin used in our experiments (DH 241 and DH 309) were responsive in anther culture and their androgenic capacity was expressed in the single-cross and three-way cross hybrids. A considerable improvement both in anther response and in the induction of haploid structures could be observed, especially in the single-cross combination between the two DH lines of Chinese origin. A similar tendency could be found for ELS formation.

In the case of the single-cross hybrid between the two DH lines there was a considerable increase in the frequency of embryo formation during the second period of culture and these embryos were able to regenerate plantlets in a relatively high number. This finding indicates that early embryos are not the only sources for haploid plant formation (Büter, personal communication). Later-formed embryos can also develop into viable plantlets under adequate culture conditions. The same phenomenon occurs on the surface of primary calli via somatic embryogenesis or organogenesis, making it possible to establish long-term regenerative haploid cultures.

While the single-cross hybrids provided a large number of regenerative structures, only a part of them were able to develop into plants. For this reason a significant difference could not be observed between the hybrids in this respect. Unfortunately a large number of regenerants was lost during transplantation into soil. The transition from a heterotrophic food supply to autotrophic assimilation is a critical step for the plantlets in *in vitro* maize cultures and a detailed study is needed to analyse the critical morphogenetical stages of differentiation. Further improvements in the *in vitro* culture conditions could help to solve this important problem.

As regards the relatively low number of fertile regenerants, it could be stated that the spontaneous doubling rate was not high in any of the genotypes studied. This resulted in a low number of doubled haploid plants which could be used for breeding purposes. Antoine-Michard and Beckert (1997) also suggested a detailed evaluation of the genetic constitution of the genotypes used for haploid induction, since there is a great difference between the responding genotypes in their ability for spontaneous chromosome doubling. Even though spontaneous doubling may exceed 40% in certain genotypes the frequency of

genetically stable doubled haploids is still very low, because of the instability of the genome. Spontaneous doubling is generally assumed to arise due to endomitosis, endoreduplication or nuclear fusion, but in some species it could also be the result of unreduced gametes (Sunderland et al., 1974; Keller and Armstrong, 1978; Wenzel et al., 1977). However, as the occurrence of spontaneous doubling is an infrequent and inconsistent event, an effective genome doubling technique is highly desirable (Loh and Ingram, 1983). The use of antimitotic drugs at the beginning of anther culture for the direct doubling of the microspore haploid genome proved to be very effective in producing genetically stable doubled haploid offspring in the case of small grain crops like wheat and rice (Barnabás et al., 1991; Alemanno and Guiderdoni, 1994). The same technique could be applied for maize, as other authors (Saisingtong et al., 1996; Antoine-Michard and Beckert 1997) and our own preliminary results indicate.

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SOILS AND AGRICULTURAL LAND USE IN TIHANY

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As the first step of the work a soil field survey was carried out. By analysing maps and laboratory data it was found that agricultural production could not be successful on lands exposed to water logging or having a shallow solum. Soils with these properties were recommended for exclusion from agricultural production.

Key words: soil, soil mapping, agriculture, sustainability, land use

Introduction

Since the beginning of agriculture, anthropogenic factors have been in effect, which means the influence of man on the natural processes of the biosphere. During agricultural production soils can be altered in ways which may hinder their further use. The planning of sustainable agriculture has to include the elimination of these harmful effects.

The planning and analysing of lands for agricultural utilisation is a complex task involving geological, geographical, climatic, biological etc. characteristics. These can be categorised by several methods, such as their effect on the economy, or the structure of their relationships with agriculture. Another aspect could be the choice and description of single features, but this way complexity is lost. Soil-forming factors contain and integrate the above-mentioned natural characteristics and the social environment affects the possibility of agricultural production through its effect on soils. The effects of the natural and social environments on each other, which can be analysed through the soils, determine the landscape.

The objective of the current research was to find a solution to the conflicts between the natural and human factors influencing agricultural production on the chosen area, by using soil maps, and to show the role of information on soils in planning sustainable agriculture.

Literature review

The Tihany Peninsula belongs to the Balaton Riviera subregion (Fig. 1). Literature evaluating the subregion and the region (Transdanubian Hills) show this area to be rich in natural values, and in agricultural, cultural and historical traditions (Marosi and Szilárd, 1975; Ádám et al., 1987–1988; Marosi and Somogyi, 1990). Though the tradition of agriculture is strong, this region became the first protected area in Hungary (Kenyeres, 1952).

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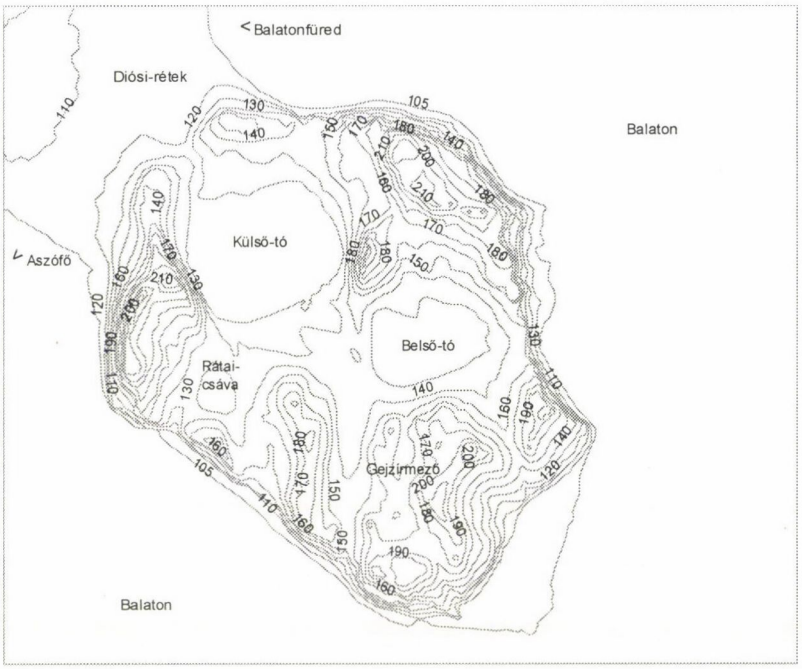

Fig. 1. Map of the Tihany Peninsula showing contour lines

Based on the natural and social characteristics and land use of the Tihany Peninsula three factors have primary importance: agricultural production, nature conservation and tourism. These factors often contradict each other. Based on analyses of the soils, the confronting interests of agriculture and nature conservation can be considered.

According to Várallyay (1994a,b), sustainable land use and agriculture must be based on soil data. The role of the soil is the following: as the most important, conditionally renewed natural resource it integrates and transforms the other natural resources, provides a site for biomass production and a storing medium for temperature, nutrients and water, and acts as a natural filter, a high capacity buffer and an important gene reservoir. Harrach deals with the connection between soil evaluation, agriculture and nature conservation (1987, 1993). The author expands the function of the soil to include the following features: it is a component in biotopes and the archives of the Earth and cultural history. Soil use adjusted to the characteristics of the site takes into consideration the cultivation of the farmland, the conservation of the traditional landscape and the conservation of soils and their ecological functions. Brady (1990) emphasizes the various possibilities for the use of soil maps. He considers that soil data are suitable for all agriculture, land evaluation and land use planning.

To sum up, soil data and soil maps are able to provide basic information for agricultural production.

Láng (1995) emphasized the importance of establishing a system of Environmentally Sensitive Areas. The basic objective is to develop a harmonious relationship between agriculture and nature conservation. The European Union proposes that environmental friendly agriculture should be subsidized. Regulated agricultural production and increased nature conservation will present the population of regions regulated in this way with new tasks and functions. The role of agriculture will be extended to cover the conservation of the farmland and of biodiversity and the prevention of environmental contamination (Harrach, 1994). Based on the examinations of several authors (Sukopp et al., 1978; Steinrücken and Harrach, 1984; Kuzmann et al., 1985) the biodiversity of soils that are not suitable for cultivation, have extreme water regimes or shallow tilth is higher than that of other soils as regards both the natural vegetation and the weeds. By excluding from production soils which are not suitable for it, results can be achieved in nature conservation as there is an inverse proportion between the agricultural value and the environmental importance of soils with respect to the growing of field crops (Harrach, 1973). Agriculture adapted to soil characteristics and functions not only promotes sustainability, but also helps in determining and conserving natural reserves.

Surveys of land suitable for agricultural production are primarily based on the discovery and analysis of features impeding soil fertility and inducing soil degradation (FAO, 1976; Dent, 1978; Schreier and Zulkifli, 1983; Jones and Thomasson, 1987; Puentes, 1987). Factors inducing soil degradation and impeding soil fertility in Hungary are summarized by Szabolcs and Várallyay (1978, 1980), who found that soils are related to society and its production system. The eight factors impeding agricultural production are the following: 1. high sand content, 2. acidity, 3. salt accumulation, 4. salt accumulation in deeper horizons, 5. high clay content, 6. water-logging, 7. erosion, 8. shallow depth.

On the basis of the above we decided not to categorize the soils in the regions chosen for evaluation, but to identify land unsuitable for cultivation from factors impeding agricultural production.

Materials and methods

Information collection and mapping were started in 1994. The soil analysis process can be divided into three parts: the reconstruction phase, the field survey and laboratory tests, and the evaluation of data (Barczi et al., 1995).

The location of sampling sites was determined with the help of earlier soil, topographic, geological and vegetation maps, complemented by points marked on aerial photos and by field experience. Three hundred and fifty points were chosen. Soil profiles were taken, according to the standards currently in force, at the points where botanical evaluation also took place (Baranyai, 1989). Samples were taken from each horizons. The samples were analysed according to the usual standards (Buzás, 1988, 1993). The analyses were primarily concentrated on general soil characteristics and the nutrition supply: organic matter, pH, total salt, clay (size: <0.002 mm) and sand (size: 0.02–2 mm) content, AL-P_2O_5, AL-K_2O, sticky point according to "Arany".

After preparing the maps data processing took place with mathematical-statistical methods (cluster analysis, distribution diagrams), analysing the maps and data simultaneously. The GIS (Geographical Information Systems) computer programmes that have been in use since the mid-1980s facilitate the handling and use of data and maps (Burrough, 1982; Mausbach and Reybold,

1987; Rao et al., 1991). Several mutually-compatible software systems are available. The ARCINFO and AUTOCAD programmes were applied in the present work. Soil types were determined according to the Hungarian classification (Stefanovits, 1992). Statistical analysis was performed according to Sváb (1967).

Land use

At the beginning of the 19th century the people of the peninsula were farming in the old, conventional way on small, poor quality plots: they produced a little of everything, mainly for their own needs (wheat and row crops) (Anon., 1959). They enlarged the area of arable land by cutting down the forests. The phylloxera disease also led to an enlargement in the area of arable land (Magyar, 1986). In 1928 Cholnoky reported that where phylloxera had destroyed the vineyards they had given way to cropland and potato fields (Cholnoky, 1928). Before the establishment of the Landscape Protection District arable lands covered a large area. With reforestation and the protection of natural resources, much of the arable land disappeared.

In Tihany animal breeding provided products only for the family. The small area of the peninsula did not allow them to keep larger livestock. Till the 19th century forest pastures played an important role in forage, leading to the denudation of the peninsula (Magyar, 1986). Today sheep-keeping is the only notable branch of animal husbandry.

Before the turn of the century the people of Tihany did not have the expertise to handle vineyards and produce wine. Their wine was of poor quality, so they made it only for their own use. In 1966 the State Farm of Badacsony, as advisors to the State Farm of Tihany, renewed the tradition of viniculture with the plantation of new red wine varieties (Oporto, Medoc, etc.) (Laposa, 1988). The vineyards around the Outer and Inner Lakes of Tihany still provide red wine, renewing the farming tradition of earlier centuries.

In earlier years vineyards and orchards were equally to be found. At the beginning of the 20th century there were more than 6000 almond trees in Tihany (Jankó, 1902). The number of fruit trees had decreased significantly by 1945. In a study on the National Park, Csordás (1947) proposed the introduction of mulberry, the establishment of distilleries and an increase in the number of fruit trees. Today, however, the importance of fruit production has decreased greatly.

At the turn of the century horticultural crops were the most profitable form of production, if also the most tiring (continuous attention, manuring, irrigation, etc.). In 1926 Bittera established a lavender plantation, which played an important role for a long time. According to Kovacsics and Ila (1988) Tihany was the most important herb producer for the pharmaceutical industry of Hungary. The improvement of horticulture was delayed after the II World War because of the lack of water. In some opinions the neighbourhood of the Inner Lake would have been suitable for horticulture (Anon., 1959).

Summarizing the above statements, it can be seen that at the turn of the century the settlement was isolated and made a living from agriculture, increasing the area of arable lands to the detriment of afforestated areas. From the 1920s, the increase in tourism brought about changes increasing the proportion of built-up areas. The number of new settlers also increased. The replanting of the areas affected by phylloxera recommenced after nationalization. Viniculture regained its earlier good reputation. The year 1952 brought another change, when Tihany became the first Landscape Protection District in Hungary. As a result of environmental and landscape protection the area of arable land and pastures decreased and the forested area increased. Harmonizing farming with the natural potential has led to many changes over the course of the century. The present less intense agricultural utilization of the area is better suited to the soil potential. Nature conservation is seriously considered in land use planning.

Results

The most important soil-forming factors on the Tihany Peninsula were found to be the parent material, the dry weather with a lack of precipitation, and the high water table. The Tihany Peninsula is located on the border between the

forest and steppe zones. The soils of this area are chernozem forest soils and brown forest soils with patches of stony soil. (The names of soil types are taken from the genetic soil map of Hungary in The National Atlas of Hungary – Magyarország Nemzeti Atlasza.) The parent material, hard basaltic tuff, determines soil formation on the belt of hills along the edge of the peninsula. Hydromorphic soils occur on the central part and the neck of the peninsula near to the lakes (Outer and Inner Lakes) or temporary ponds (Rátai-csáva). Drier soils with deeper horizons are located on more or less flat areas on the remaining parts. If the soil types are projected onto the TIN (Triangulated Irregular Network) topographic map it can be observed that the stony soils are located on the hill belt, while the lakes are surrounded by a ring of hydromorphic soils (Fig. 2).

According to topographic maps the Diósi meadows near Aszófő are located on the low-lying area between the Balaton Hills and the higher-lying areas of the Tihany Peninsula. Here the high water table has greater importance in soil genesis, as the land is often covered with water. On higher topographic positions with higher slope angles the soils are drier but are more subject to erosion and have a less deep solum. Stony soils were found on the small plateau where solid rock is near to the surface. Due to erosion and mechanical cultivation, stony soils and colluvial deposit soils are abundant. The rock of which the hills are formed is the result of former local volcanic activity. There are mixed colluvial soils at the bottom of the slopes both on the northern and the southern side.

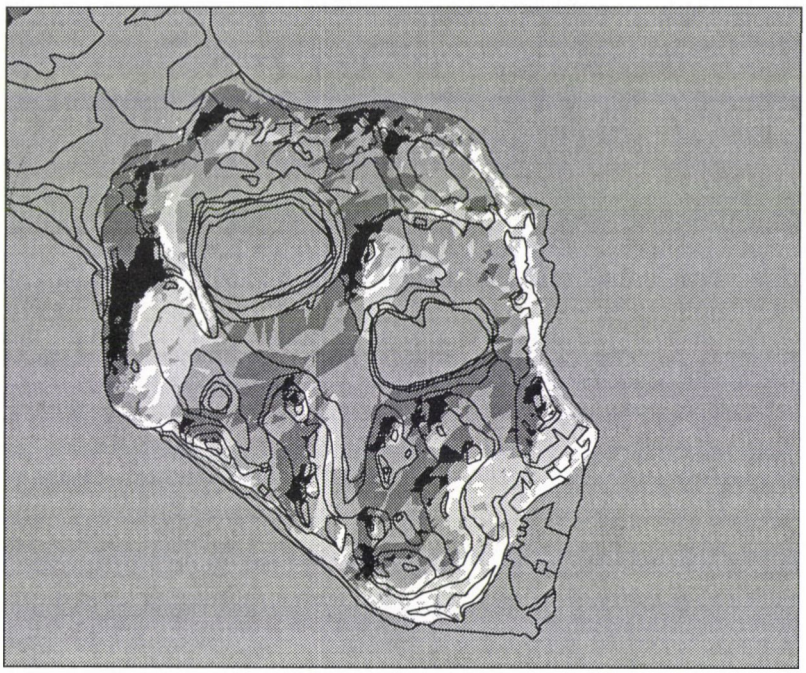

Fig. 2. The TIN map showing the borders of the soil types

The soils are located in belts around the Outer and Inner Lakes due to the basin effect. As we approach the lake the water table is closer to the surface. There is a similar phenomenon southeast of the lake at the temporary pond (Rátai-csáva). The area is surrounded by shallow (10-40 cm deep) soil formed on tuff.

There are chernozem brown forest soils east of the belt of shallow soils and on a great patch south of the Outer Lake on the central area of the peninsula, which is farmed, as it is the most valuable soil from the agricultural point of view.

The patchiest part of the peninsula is the Geyser Basin. The peaked tops of the geysers are covered with stony soil, while rendzinas occur on the area between them and colluvial deposit soils can be found in the accumulation zone. The small rock outcrops and the stony soils covering them could not be presented on the map.

The proportions of the various soils covering the area is summarized in Table 1.

Table 1
The percentage of different soils covering the surface
(including the FAO soil types)

Soil type	%
Anthropogenic soils, urban area	12.09
Stony and rocky soils (Leptosols)	33.88
Brown forest soils (Cambisols)	13.04
Meadow soils (Vertisols)	11.28
Peaty, alluvial, colluvial deposit soils (Fluvisols, Gleysols & Histosols)	26.81
Open water surface, lake	2.90

As mentioned in the literary review, there are eight factors that prevent the agricultural utilization (in this case grape or crop growing) of the land in Hungary. All the factors are based on the extreme value of one soil characteristic; high sand and clay contents, for instance, are extremes of texture. Salinization in the top or deeper horizons and acidity are extremes of salt content or pH, water-logging shows an extreme moisture regime. Shallow horizons can be correlated with erosion susceptibility.

Seventeen soil profiles were examined and sampling took place at 63 locations, so samples from a total of 80 locations were analysed. The most important soil properties were examined, such as texture, pH, nutrient content, etc. and emphasis was placed on tests connected to factors impeding soil fertility. First the homogeneity of the soil types was tested by comparing properties with cluster analysis. Then normal distribution diagrams were drawn from the results of the tests in order to determine the scale of extremes in the soil mantle of the area.

There were no extreme values for pH, clay content or sand content (Fig. 3), so these features are not characteristic of the soils of the Tihany Peninsula.

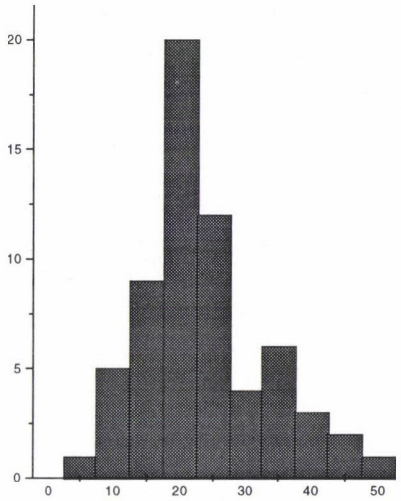

Fig. 3. Sand content (size: 0.02-2 mm) distribution diagram (%)

There are more extreme values and higher distribution in total salt content, indicating extremes in salt content, but the measured values are below the limit value of salinization (0.1%) (Fig. 4).

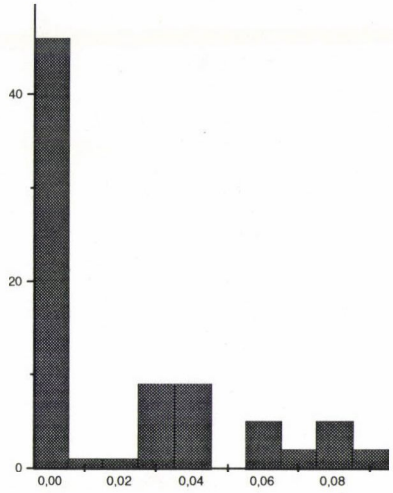

Fig. 4. Total salt content distribution diagram (%)

Based on these results, high clay and sand contents, salinity and acidity (pH <5.5) can be excluded from the factors preventing agricultural production here. However, extreme water regime, shallow solum (Fig. 5), slope and erosion susceptibility are significant fertility-reducing factors in the soil mantle.

Using the percentages of the different soil types (see Table 1) factors preventing agricultural utilization, such as shallow solum, erosion susceptibility and water logging, can be determined and drawn on a map (Fig. 6).

Water-logged territories are located on the shoreline, around the lakes, and at the neck of the peninsula. Shallow solum and high slope angle, causing erosion susceptibility, are characteristic of several parts, but occur primarily on the belt of hills and on the basaltic tuff saturated with silicic acid or lime in the Geyser Basin. High slope angle occurs on the belt of hills and is less important on the geyser cones. These areas are not suitable for farming (growing grapes or crops). The map shows that only a limited area is suitable for agricultural utilization.

There are no limiting factors on the area covered by chernozem brown forest soils with small slope angle in the centre of the peninsula. The area north of the pond (Rátai-csáva) can also be utilized. The forest soils which have developed on the hillslopes with small slope angle would be suitable for agricultural production, but these areas have forest vegetation. It should be emphasized that the purpose of this work was not to recommend the agricultural utilisation of this land, but to eliminate unsuitable areas from agriculture.

When the polygons were drawn, the borders for possible landuse were not marked, because it was felt that the nature conservation policies should have priority.

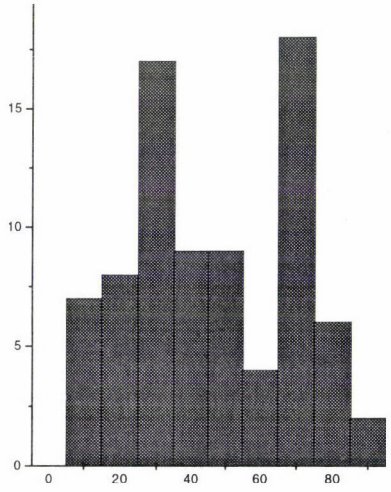

Fig. 5. Distribution diagram for the total thickness of the A and B horizons (cm)

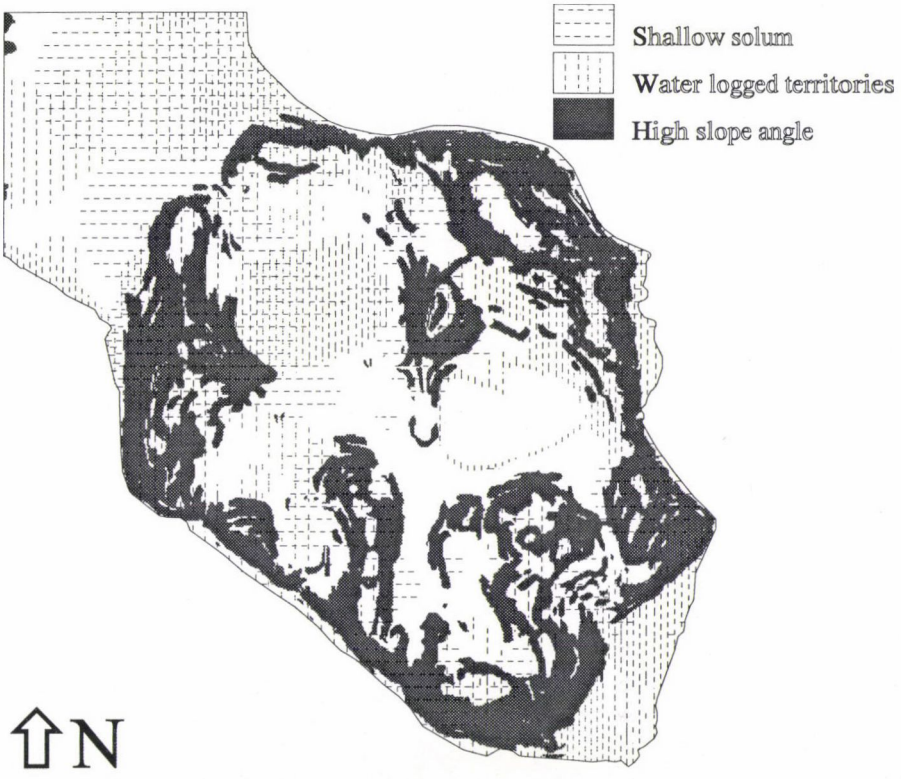

Fig. 6. Lands not suitable for agricultural utilization

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NEW FORMS OF COMPLEX ORGANOMINERAL FERTILIZERS AS A FACTOR FOR SUSTAINABLE DEVELOPMENT OF BIOSPHERE AND PLANT PRODUCTIVITY

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The composition and nutrient ratio of new forms of complex organomineral fertilizers are based on the metabolic needs of cereal and vegetable crops. These fertilizers comprise the following main macroelements (N, P, S, K, Ca, Mg), microelements (Mo, Mn, Cu, Li, Zn), physiologically active substances and humus-like compounds. The latter stimulate the growth of the root system and possess high complex-forming activity. The application of these fertilizers to spring wheat (*Triticum aestivum* L.) and beetroot (*Beta vulgaris* L.) gives rise to directed changes in plant architecture, which strengthen the source-sink relationships between the plant organs and stimulate their functional activity. This reinforces the uptake and assimilation of mineral elements, particularly nitrogen. The above changes in morphological structure lead to the intensification and realization of plant biological potential as well as to an increase in yield and an improvement in its quality.

Key words: biosphere, functional activity of plant organs, humus, hydrolytic lignin, morphological structure of plants, nitrogen, nitrate reductase activity, radionuclides, root system, source-sink relationships, yield quality

Introduction

A review of numerous papers proves that the correct application of mineral fertilizers together with growth regulators, herbicides, fungicides and other compounds allows high yields of crops with improved quality to be obtained (Heinisch et al., 1979). Some 50–60% of the total yield surplus is achieved due to mineral fertilizers. The remainder is distributed as follows: 20–25% as the result of new varieties and hybrids, and 20–25% by means of improvements in growing technologies (Tyutyunikov, 1991).

The constant desire to achieve maximum yields led to the development of intensive (industrial) crop production technologies based on the utilization of high doses of mineral fertilizers, growth regulators, herbicides and pesticides. However, these technologies became highly energy-intensive and ecologically dangerous, and did not always cover their expenses, because of the need for their frequent application during the vegetation period and the great losses of agrochemicals into the environment (sometimes 50–90%) (Zhuchenko, 1990).

The use of specialized, genetically uniform varieties and agroecosystems in these technologies inhibited the natural ability of the crops to achieve ecological balance in the biosphere through the mechanism of self-regulation.

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This is why chemical agents, applied in intensive technologies, are constantly accumulated in the biosphere and have a negative influence on the processes of sustainable development.

Moreover, the intensive use of atomic energy in different areas of the national economy, as well as the vigorous development of industry and transport, has resulted in the contamination of the biosphere, particularly the arable layer of the soil, with radionuclides and heavy metals. These agents, being absorbed by plants and accumulated in plant production, are a serious threat to human and animal health.

Taking into consideration the above, much attention has been paid in recent years to the development of new technologies, which it is hoped will lead to a second "green revolution" (Mirkin et al., 1991). The main distinction between the future technologies and those used at present should be moderate energy consumption, ecological safety and the creation of favourable soil conditions, contributing to high quality stable yields and an increase in soil humus content and fertility.

According to some authors the basis for future technologies will be the activation of the biological resources in the plant-soil system by providing plants with nutrients and physiologically active substances during their ontogenesis, and the creation of favourable conditions in the soil for plant growth and development. These conditions include: 1) the location and fixation of nutrients in the root area; 2) the regulation of the pH of the soil solution; 3) the saturation of the soil-absorbing complex by bases; 4) improvements in the physico-chemical structure and aeration of the soil; 5) an increase in soil humus content and its fertility. The components of this system (plant and soil) are closely interconnected, so they will be profoundly influenced by these technologies. By altering a number of factors (nutritional background, water regime, application of growth regulators, etc.), it is possible to regulate biochemical and physiological processes directly and thus to control the development of the system. Since the soil is the habitat of the roots and is under the control of the nutritional background, it will have a direct effect on the root system. The latter is a central part of the system of morphostructure regulation (Klimashevskaya, 1986). Many researchers consider that the morphostructure of the plant plays a leading role in the development of plant productivity, which is why its control is very important in the achievement of genotype potential (Shulgin et al., 1986).

It can be concluded from the above that creating a specific mineral and organic nutrient background and regulating with its help the soil conditions makes it possible to form the plant architecture necessary to obtain large yields of high quality. Obviously, stimulation of the forming and growing processes of the root system allows it to enlarge its share in the total plant biomass. As a result of this, the source-sink relationships between the organs will act as a trigger mechanism, intensifying the functional activity of both the root system and the aboveground plant parts.

Consequently, the activation of source-sink relationships by stimulating the growth of the root system through the creation of a satisfactory nutrient background will lead to the intensified uptake of nutrients by the roots and their assimilation in plant tissues. This in its turn will cause an increase in the effectiveness of the agrochemicals applied and a decrease in chemical losses. The regulation of plant architecture and an increase in the functional activity of plant organs will thus help to protect the environment from pollution by agrochemicals.

The above-mentioned changes in the plant-soil system will make it possible to reduce both the rates of agrochemicals applied and the proportion of agrochemical methods in new technologies. It will result in a decrease in their energy intensity, a cut in production costs, and a restriction of the anthropogenic stress caused to the biosphere. Regulating plant architecture and strengthening the functional activity of plant organs in the plant-soil system will bring about a reinforcement of the processes of self-regulation and adaptation. This will favour the sustainable development of the biosphere.

Thus, the plant-soil system, which occupies a central place in the cycle of processes regulating biosphere development and is dependent on anthropogenic stress, exerts a decisive influence on the biosphere. In this connection it is probable that the basis of the new intensive technologies will be new forms of complex organomineral fertilizers with a slow release of components during plant development. It is thus time to develop forms of fertilizers containing not just one or two nutrients but a whole complex of the main macro- and microelements, combined with organic and physiologically active substances. If the constituents of these complexes are in optimal ratio, they will resemble natural substances such as humus, manure and green manure and will have a favourable effect both on plants and soil, thus serving to regulate both the formation of the necessary plant architecture and the physico-chemical processes in the soil. Their application would lead to a reduction in agrochemical doses and would diminish the amount of agrochemicals required in new technologies. The presence of organic substances capable of complex formation would make it possible to fix and accumulate minerals in the root zone and to increase the organic matter content and fertility of the soil. However, when determining the ratios of the various components in these fertilizers the different metabolisms of cereal and vegetable crops should be taken into account. For example, to increase the yield and the protein and gluten contents of wheat grains, nitrogen must predominate over the other elements in the NPK ratio. On the other hand, to raise the sugar content of fruit and root crops, to increase the starch content of tuber crops, and to limit the accumulation of nitrous compounds, phosphorus and potassium must predominate over nitrogen in the organomineral fertilizer intended for vegetables. Consequently, the aim of the present work was to develop complex organomineral fertilizers and to study their influence on the growth of cereal and vegetable crops, with special regard to an increase in productivity and an improvement in quality.

Materials and methods

Organomineral complexes were used to control the architecture of cereal plants and vegetable crops and to increase their productivity. These complexes were based on hydrolytic lignin (HL). The latter was subjected to chemical modification by nitrated oxidation using nascent nitric acid. As a result of reactions such as oxidation, fission and nitration inert macromolecules of hydrolytic lignin were converted into oligomeric humus-like substances, containing a large number of functional groups, particularly carboxyls and phenolhydroxyls (Fig. 1).

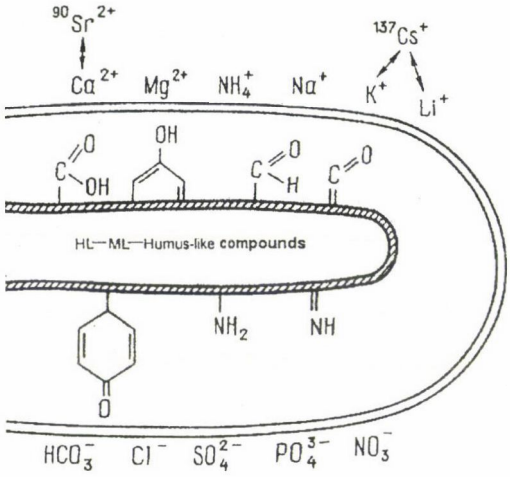
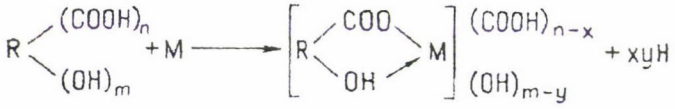
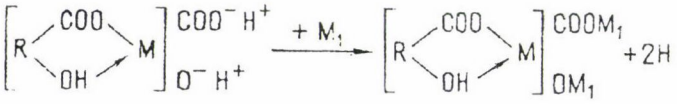


Fig. 1. Humus-like compounds, their functional groups and complex-forming ability

These compounds, like humus acids, not only exhibit the properties of physiologically active substances, stimulating root formation and the growth of the root system, but also possess complex-forming ability. Modified lignin interacts with salts containing N, P, S, K, Ca, Mg, Mo, Mn, Cu, Zn and Li, and with physiologically active substances such as chlorocholine chloride and DMSO, to form organomineral complexes or complex-hetero polar salts (Fig. 2), which slowly release nutrients. These complexes are considered to be effective, slow-acting organomineral fertilizers (Lyaskovsky et al., 1996; Lyaskovsky, 1997).



where: **M – $Fe(OH)^+_2$; $Fe(OH)^{2+}$**



where: **M– $Fe(OH)^+_2$;$Fe(OH)^{2+}$**

M_1 – Ca, Mg, K, Na, Li, Mn, Mo, ^{90}Sr, ^{137}Cs, Cu, Zn, etc.

*Fig. 2.*Complex-heteropolar salts (Aleksandrova, 1980)

Two forms of complex organomineral fertilizers were developed to suit the metabolisms of cereal and vegetable crops: form N 1 for cereal plants, with an NPK ratio of 1.5:1:1, and form N 2 for vegetable crops, with an NPK ratio 1:1.5:2. There was virtually no difference in the contents of other elements (Ca, Mg, S, Mo, Mn, physiologically active and humus-like substances), though the form intended for vegetables also contained Li, Zn and Cu. The effectiveness of these fertilizers was studied over a number of years on a number of different plants. In order to trace their influence on the morphological structure, productivity and yield quality of cereal crops and on the accumulation of radionuclides in root crops and vegetative plant mass, the crops spring wheat (*Triticum aestivum* L. cv Saratovskaya 29) and beetroot (*Beta vulgaris* L. cv Bordo) were chosen.

Spring wheat cv Saratovskaya 29 has a long thin stem with narrow leaf blades and low protein content. These plants can be used as a highly sensitive natural model for following both the influence of organomineral fertilizer on morphological structure and the effect of the latter on plant productivity and grain quality. In the case of food root crops, carrot and beetroots, for example, accumulate a large quantity of radionuclides and nitrates, so plants of beetroot cv Bordo could be a good model for studying the influence of organomineral fertilizers not only on the processes of yield forming and its quality, but also on radionuclide accumulation in the tissues.

The plants were grown in Vagner's vegetative pots with a capacity of 8 kg soil in the greenhouse of the Institute of Plant Physiology and Genetics of the National Academy of Sciences of the Ukraine. The experiments were replicated 20 times. The pots were filled with podzolized meadow chernozem soil selected from the fields of the Experimental Research Base of the National Academy of Sciences of the Ukraine. It was contaminated with different amounts of radionuclides. The contents of ^{90}Sr and ^{137}Cs were 81 and 307 Bq/kg in the experiment with spring wheat cv Saratovskaya 29, and 360 and 520 Bq/kg in the experiment with beetroot cv Bordo. The fertilizers were added to the soil during pot filling. A mineral mixture consisting of 4.7 g nitroammonium phosphate fertilizer and 0.8 g urea was added to the soil of the control pots when growing spring wheat. This concentration was equal to an amount of active substances per pot of $N_{1.2g}$: $P_{0.8g}$: $K_{0.8g}$ with an NPK ratio of 1.5:1:1. Eighteen g of complex slow-acting organomineral fertilizer (CSAOMF, form N 1) was added to the soil of the experimental plants. This dose corresponded to the same amount of nutrient substances and to the same NPK ratio as in the control pots. The content of modified hydrolytic lignin (ML) in the organomineral fertilizers was 17% of the weight of the mineral part. Two more variants were included in the scheme of experiments to clarify the role of the organic component in the fertilizer. The organic component was absent in one variant. The fertilizer comprised only macro- and microelements and physiologically active substances. This fertilizer was referred to as a complex slow-acting mineral fertilizer (CSAMF). In contrast to this a complex organomineral fertilizer, containing 30% modified lignin, was used in the second variant. For growing beetroot cv Bordo, a mineral mixture consisting of 4.7 g nitroammonium phosphate fertilizer, 1.57 g monopotassium hydrogen phosphate (KH_2PO_4) and 0.7 g potassium chloride (KCl) was added to the soil of the control pots. This dose of fertilizer corresponded to the following amount of active substances per pot: $N_{0.8g}$: $P_{1.2g}$: $K_{1.6g}$ and to an NPK ratio of 1:1.5:2. Then 28 g of complex slow-acting organomineral fertilizer (CSAOMF, form N2) were added to the soil of the experimental variants. The element contents and NPK ratio in this amount of fertilizer was similar to that in the control pots.

Thus, the control and experimental variants were equal only in the content of NPK. The other constituent parts of the complex organomineral fertilizer (physiologically active and humus-like compounds, macro- and microelements) were absent in the control pots. It was this multi-component composition that gave the new forms of fertilizers their advantage over the traditional ones applied in the control pots.

Twenty-five seeds of spring wheat and 20 seeds of beetroot were sown in the soil-filled pots. After sprouting and tillering the wheat plants were thinned out, leaving only 20 in each pot. When the second true leaf of beetroot appeared, these plants were also thinned out. At first three plants were left, while after a month only one plant was left in the pot.

The influence of complex organomineral fertilizer on the formation of the spring wheat root system and on the architecture of the plants was studied during ontogenesis. For this purpose

3 pots of each variant were selected on four occasions (at heading, flowering, beginning of grain ripeness and milky-waxy ripening) and their roots were washed clean. Then the roots and shoots were separated and the root mass, root volume and shoot mass were measured, after which the plants were divided into organs (flag-leaf, stem and roots). The plant material was chopped finely, fixed at a temperature of 105°C for 20 min and dried at 45°C till air-dry. The nitrate reductase activity was studied in the fresh material for each plant organ. The contents of total, albuminous and mineral nitrogen were measured in the dry matter. At the end of the vegetation the remaining plants were cut and the morphological structure of the plant culm and the yield were studied. The quantities of protein, gluten and amino acids in the grain were determined.

Beetroot was harvested late in September. The beet tops were separated from the root crops. Both parts of the plant were measured, chopped finely and, after fixation at 105°C for 20 min, dried at 45°C till air-dry. The sugar, nitrate and dry matter contents were determined in the fresh material.

The ^{90}Sr content in wheat straw and grain and in beetroot tops and root crops was determined radiochemically and the ^{137}Cs content using a spectrometer AM-A-O2 F1 (Gudkov, 1991). The results were statistically analysed.

Results

While analysing the morphological structure of spring wheat plants during their vegetation changes were observed caused by the composition of the organomineral fertilizer. The experimental plants differed from the control in having shorter, thicker culms, wider leaf blades of dark green colour and a well-developed root system. These changes were stable during ontogenesis. Within a few years they were revealed so dramatically that the plants lost their variety features.

Root system formation and root:shoot ratio

An inverse relationship between the growth intensity of the aboveground plant parts and the root system was revealed when studying root system development and the root:shoot ratio. The lower initial shoot growth intensity of variants treated with organomineral fertilizer thus stimulated the development of the root system. Later on, the increase in the amount of aboveground plant mass was accompanied by an inhibition of the root system, which reached its maximum value at the heading stage, after which the dying off of the roots predominated over their formation. As the result of this, at the stage of milky-waxy ripening the root mass was greatly diminished, being only 32% of the maximum value observed at the heading stage in the control plants. In the experimental plants this value was 50–52%.

The dying off of the root system after the heading stage caused a reduction in the root:shoot ratio. Thus, the differences in the architecture of the aboveground parts of spring wheat which had developed by the heading stage indicated that the complex organomineral fertilizer slightly retarded the growth of the aboveground mass (5–7%), thus significantly intensifying the growth of the root system (15–20%) and causing an increase in the root:shoot ratio (20–30%) (Table 1).

Table 1
Formation of the root system and root:shoot ratio in spring wheat cv Saratovskaya 29 during ontogenesis

Index investigated	Variants			
	Control	CSAMF	CSAOMF (17% ML)	CSAOMF (30% ML)
		Heading		
Aboveground mass,	143.3±12.5*	135.8±9.6	132.5±7.8	135.9±10.2
g per pot	100	95	93	95
Root weight,	58.6±3.9	64.3±7.3	67.2±4.7	68.7±3.8
g per pot	100	110	115	120
Root:shoot ratio	0.41±0.08	0.47±0.05	0.51±0.04	0.50±0.03
	100	118	128	122
		Flowering		
Aboveground mass,	154.9±7.8	158.0±11.5	164.9±14.9	175.0±10.6
g per pot	100	102	106	113
Root weight,	35.5±3.7	48.7±4.2	57.5±6.90	56.0±7.2
g per pot	100	137	162	158
Root:shoot ratio	0.23±0.2	0.31±0.06	0.35±0.02	0.32±0.02
	100	135	152	140
		Beginning of grain ripening		
Aboveground mass,	193.3±17.5	195.8±12.7	163.9±9.7	167.7±11.3
g per pot	100	101	85	87
Root weight,	23.2±1.4	37.2±0.8	45.9±4.3	43.6±2.9
g per pot	100	160	198	188
Root:shoot ratio	0.12±0.02	0.19±0.02	0.28±0.03	0.26±0.027
	100	158	233	217
		Milky-waxy ripening		
Aboveground mass,	185.7±12.9	190.4±13.7	185.3±16.7	191.1±17.0
g per pot	100	102	100	103
Root weight,	18.5±1.9	26.7±2.8	35.2±2.7	34.4±3.8
g per pot	100	144	190	186
Root:shoot ratio	0.10±0.01	0.14±0.02	0.19±0.01	0.18±0.00
	100	140	190	180

*Note: Above the line - absolute quantity; below the line - as a percentage. The same notation is used in Tables 2, 3 and 5.

The distinctions observed at the heading stage between the variants with respect to vegetative mass accumulation gradually disappeared by later stages of plant development. However, the differences became more significant in the mass of the root system. The root mass of the experimental plants exceeded that of control plants by 40–50% during flowering. At the beginning of grain formation and at milky-waxy ripening it was higher by 88 and 98%, respectively. The mixture containing mineral components and physiologically active substances without organic substances (variant CSAMF) also stimulated the growth of the root system, though the increase in the root system was

significantly lower than in the case of organomineral fertilizer. An increase in the organic component in the fertilizer to 30% gave no additional stimulation to the root system. The root system growth rate was equal to that observed for the optimum amount of modified hydrolytic lignin in the fertilizer (17%). It is obvious that the ratio of organic and mineral components in the fertilizer is optimum when the amount of hydrolytic modified lignin is 15–20% and any deviation away from this figure causes a decrease in fertilizer efficiency.

Thus, by stimulating root formation and growth, complex organomineral fertilizer is responsible for the regulation of wheat plant architecture: the inhibition of shoot growth and the strengthening of the root system. Moreover, the hydrolytic modified lignin component not only stimulates the growth of the root system, but also contributes to a retardation of aging, allowing it to function for a longer period. As a result of this, the root:shoot ratio in the experimental plants significantly exceeded that of the control plants during vegetation. This was especially obvious during ripening and was caused by the rapid dying off of the roots in the control plants.

Dynamics of nitrogen content in wheat plant organs

By increasing the root:shoot ratio and intensifying the functional activity of the root system, complex organomineral fertilizer resulted in greater nitrogen uptake and accumulation in the experimental plants (Fig. 3).

The maximum quantity of N was accumulated in the flag-leaf, stem and roots at the heading stage, when root system development was at its peak. By the end of the vegetation the nitrogen content in the investigated organs decreased due to the reutilization of the nitrogenous substances accumulated in the vegetative mass. At the flowering stage the process of nitrogen reutilization proceeded more actively in the culms and roots, whereas in the flag-leaf the N content diminished by only 8%, as compared to 41% in the stem and 30% in the roots (Fig. 3). This made it possible for the leaf to preserve a high intensity of CO_2 assimilation for some time.

The reutilization of nitrogenous substances increased as the grain matured. By the phase of milky-waxy ripening 58% of the nitrogen accumulated in the flag-leaf at the heading stage, 76% of that accumulated in the stem and 52% of that in the roots had been used up.

By delaying the aging of the root system and stimulating root formation, organomineral fertilizer favoured mineral nitrogen uptake by the roots during plant reproductive development. As a result of this, at the flowering stage the nitrogen content was twice as high in the stem and 18–20% higher in the flag-leaf and the roots than in the control plants. In later stages of plant development these differences increased: at the beginning of grain maturing the nitrogen content was 2.5 times higher in the stem and 27–39% and 38–58% in the flag-leaf and roots, respectively, than in the corresponding organs of the control plants. At the stage of milky-waxy ripening these differences were 3–3.5 times, 66–74% and 27–42%, respectively (Table 2). The lower intensity of nitrogen reutilization in the experimental plants can be attributed to the fact that the nitrogen requirements of the reproductive organs were mainly satisfied at the expense of the mineral nitrogen fairly intensively absorbed by the roots during this period of development.

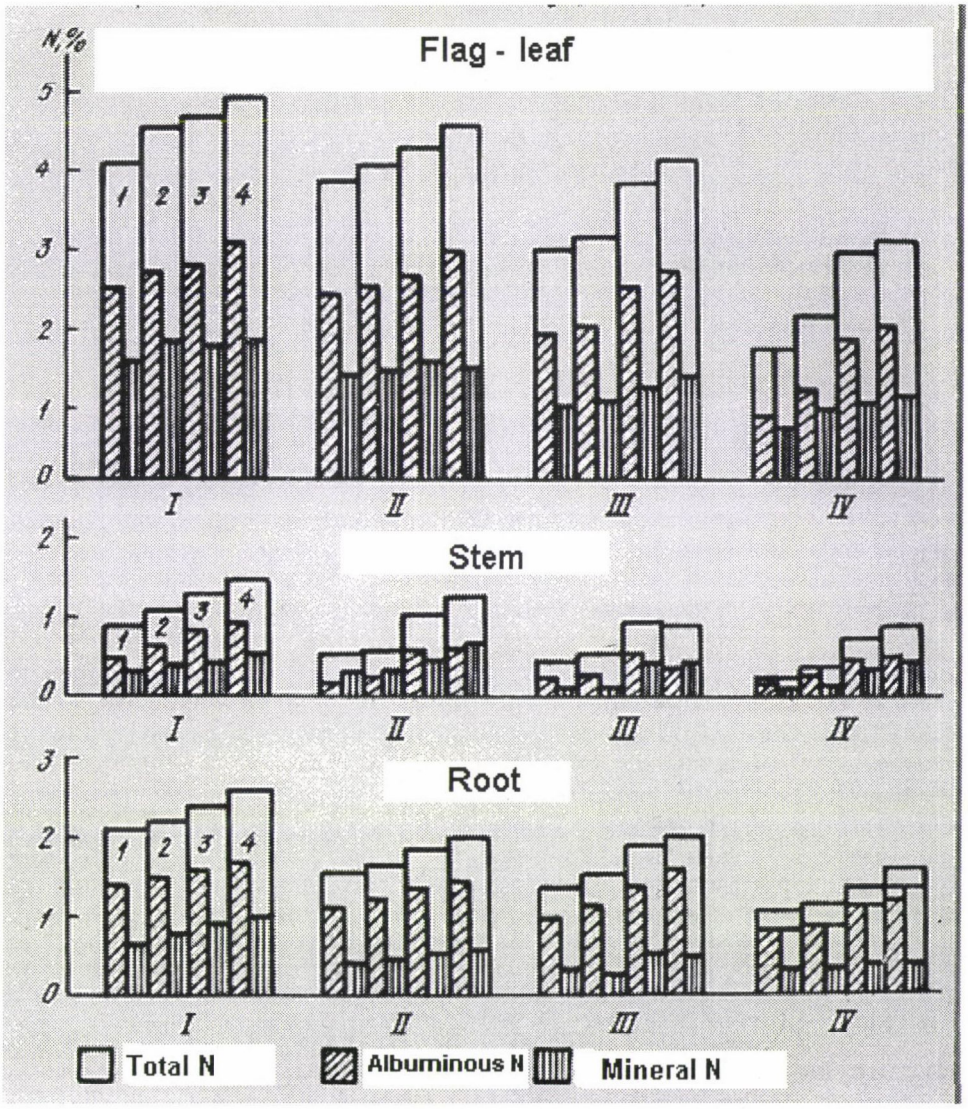


Fig. 3. Dynamics of different forms of nitrogen in spring wheat cv Saratovskaya 29 (% in air-dry mass).
Variants: 1 - control, 2 - CSAMF, 3 - CSAOMF (17% ML), 4 - CSAOMF (30% ML).
Stages of plant development: I - heading, II - flowering, III - beginning of grain ripening, IV - milky-waxy ripening.

Thus, a comparison of the dynamics of nitrogen accumulation in the organs investigated with the intensity of root system development indicates the existence of a close interrelationship between these factors, detectable in all the variants though expressed to different extents. The greater the root system stimulation and the increase in the root:shoot ratio, the more significant was the amount of nitrogen accumulated in the tissues. However, as previously noted, an increase in the organic component of the fertilizer to 30% by weight of the mineral part resulted neither in additional stimulation of root system growth as compared to the optimum variant (17% ML), nor in an increase in the nitrogen accumulated in the tissues. The total nitrogen amount, like the root mass in this variant, was slightly lower as compared with the optimum variant.

Nitrate reductase activity and its distribution within wheat plant organs

Nitrate reductase (NR) is known to be a key enzyme in the nitrogen metabolism (Ismailov, 1986), so it is interesting to follow the influence of organomineral fertilizer both on the activity of this enzyme and on its distribution in different organs during wheat plant ontogenesis. A high level of enzyme activity was found in the flag-leaf and roots in the flowering stage (Fig. 2). In the stem, the ear and under the flag-leaf the enzyme activity was either insignificant or was not detected at all. At the end of flowering the nitrate reductase activity was distributed approximately equally between the root and the flag-leaf, being 52% and 48%, respectively. In the experimental plants, on the other hand, 68% of the enzyme activity was concentrated in the flag-leaf and only 18% in the roots, while nitrate reductase activity was also observed under the flag-leaf (9%) and in the ear internode (5%). The enzyme activity in these organs was absent in the control plants.

In the period of milky-waxy ripening the highest level of nitrate reductase activity was recorded in the roots, with only half this quantity in the flag-leaf. In the other wheat plant organs, except the ear, nitrate reductase activity could not be measured at all. The nitrate reductase activity in the roots of the control plants was 67.7%, in the flag-leaf 25.9% and in the ear 6.4%. In the experimental plants these figures were 59.2, 31.4 and 9.5%, respectively (Fig. 4).

The study of nitrate reductase activity in the flag-leaf during plant vegetation showed that it was maximum in the heading stage and as the plants passed into the phase of reproductive development the enzyme activity was reduced to a minimum (Fig. 5A). By contrast, during plant vegetation the nitrate reductase activity in the roots increased, obviously compensating for the decrease in the flag-leaf (Fig. 5B). Organomineral fertilizer (with the optimum 17% content of modified hydrolytic lignin) enhanced the nitrogen uptake of the plants, thus inducing NR activity in their tissues. In the phases of heading and flowering the enzyme activity in the flag-leaf increased by as much as 2–3 times in comparison with the control plants. At the beginning of grain ripening and at milky-waxy ripening it was 30 and 50% higher, respectively. The enzyme activity in the roots was also stimulated by organomineral fertilizer. Complex slow-acting mineral fertilizer (CSAMF) induced nitrate reductase activity both in the flag-leaf and in the roots.

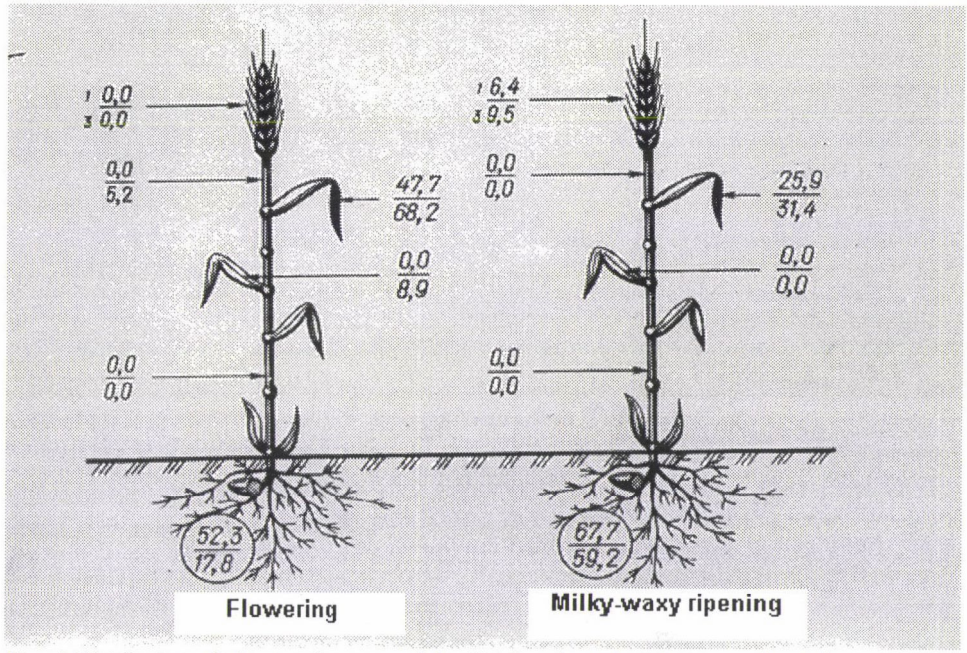


Fig. 4. Distribution of nitrate reductase activity (% of total amount) between wheat plant organs. Above the line - control, below the line - CSAOMF (17% ML).

μg NO_2^- • 30min $^{-1}g^{-1}$ fresh weight

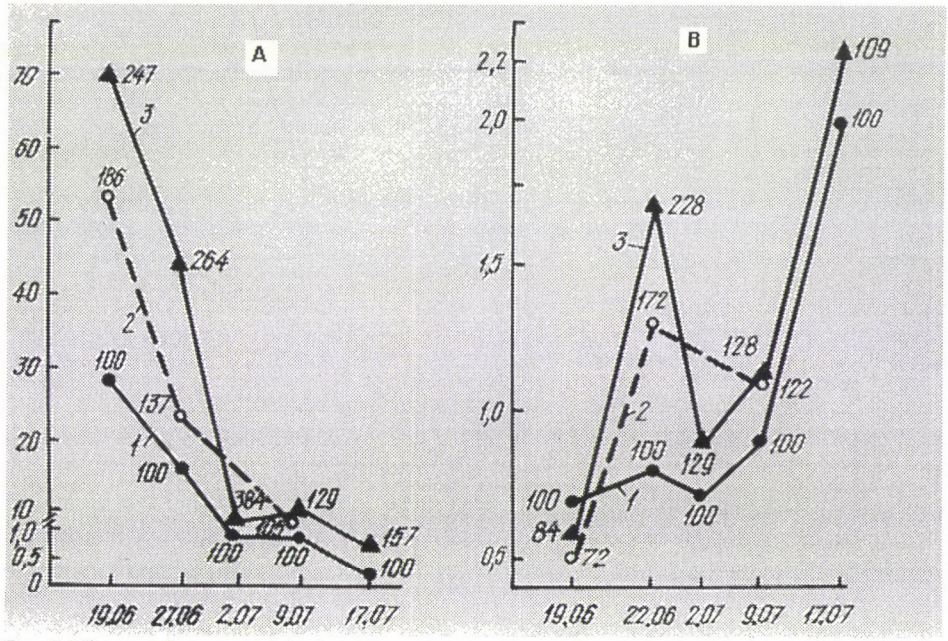


Fig. 5. Nitrate reductase activity in spring wheat cv Saratovskaya 29. A - flag-leaf; B - root system. Indication of variants is the same as in Fig. 3.

However, the presence of modified lignin in the mineral mixture led to even greater enzyme activity. Thus, by stimulating the growth of the root system organomineral fertilizer improved both the root:shoot ratio and the nitrate reductase activity, creating favourable conditions for the assimilation of the mineral nitrogen absorbed by the roots. It also increased nitrogen accumulation within the plant tissues in the form of nitrogenous compounds.

Morphological structure of spring wheat stems

The components of complex organomineral fertilizer gave rise to significant changes in plant architecture (Table 2). The height of the experimental plants was 24–27% lower, while the stems were 20% thicker. The ratio of stem diameter (d) to height (h), which is an indicator of plant resistance to lodging, rose by 58–62%. It is known that an increase in the root:shoot ratio contributes to the development of stem anatomical structure, in particular mechanical tissues, leading to improved resistance to lodging (Prusakova, 1985; Lyaskovsky, 1991a). Consequently, by inhibiting the upward growth of the stem and stimulating root system development, the components of complex organomineral fertilizer were responsible for changing the architecture of spring wheat, increasing resistance to both plant lodging and unfavourable factors of the environment.

Table 2
Formation of morphological structure in stems of spring wheat cv. Saratovskaya 29

Index of stem morphostructure	Variants			
	Control	CSAMF	CSAOMF(17% ML)	CSAOMF(30% ML)
Stem height, (h), cm	112.3±3.2	82.4±3.6	85.5±1.3	84.3±3.1
	100	73	76	75
Diameter (d) of the second internode, mm	2.71±0.08	3.21±0.05	3.24±0.05	3.27±0.09
	100	119	120	121
Ratio d/h	0.024	0.039	0.038	0.039
	100	162	158	162
Strength of the second internode, g/cm	1073±126	1190±112	1257±107	1249±157
	100	111	117	116

Yield of spring wheat and grain quality

By changing the plant morphological structure and by increasing the functional activity and prolonging the vital activity of the plant organs, organomineral fertilizer created favourable conditions for the development of yield structural elements and the deposition of reserve substances in the grain (Table 3). It resulted in increases of 40–48% in grain yield, 34–36% in protein content, 39–47% in wet gluten and 33–40% in total and essential amino acids in the grain. The mineral mixture (CSAMF) also contributed to a yield increase and to the improvement of its quality. However, the addition of the organic component (ML) to the mineral mixture caused an increase in the effectiveness

of the fertilizer. The enhancing of the modified lignin content in the fertilizer to 30% gave rise to neither a further increase in yield, nor to an improvement in quality as compared with the optimum variant.

Thus, being responsible for the directed regulation of plant morphological structure and the intensification of the functional activity of the organs, the components of organomineral fertilizer contributed to the realization of plant biological potential, leading to an increase in grain yield and an improvement in grain quality.

Table 3

Effect of CSAOMF on yield and grain quality of spring wheat cv Saratovskaya 29

Index investigated	Variants			
	Control	CSAMF	CSAOMF (17% ML)	CSAOMF(30% ML)
Grain mass, g/pot*	22.1±2.3 100	26.1±1.7 118	32.7±2.6 148	30.9±2.3 140
Protein content, %	13.5±0.3 100	15.4±0.2 114	18.4±0.5 136	18.1±0.6 134
Total amount of amino acids, mg/100 g flour	11.5±0.6 100	12.1±0.2 105	16.1±0.4 140	15.3±0.2 133
Content of wet gluten, %	21.0±0.5 100	26.2±0.4 125	30.9±0.4 147	29.2±0.5 139

*$LSD_{0.05}$= 2.67; $LSD_{0.05}$= 9.23

Accumulation of ^{90}Sr and ^{137}Cs in wheat plants

It could be assumed that strengthening the functional activity of the plant organs, and of the root system in particular, under the influence of organomineral fertilizer might result in the accumulation of harmful elements (radionuclides, heavy metals) in plant organs. This must be taken into account when cultivating farm crops on ecologically polluted areas. Another series of experiments was thus carried out to study the influence of organomineral fertilizer on the radionuclide accumulation in plants. The experiments were conducted on the cereals winter and spring wheat, oats and barley (Lyaskovsky et al., 1995).

The study of ^{90}Sr and ^{137}Cs accumulation in spring wheat (cv Saratovskaya 29) showed that the mineral mixture (CSAMF) reduced the amount of radionuclides in plants by more than 2.5 times as compared with the control plants (Table 4). When 17% modified lignin was added to this mineral mixture (CSAOMF), it contributed to raising the yield and its quality, as well as to reducing the radionuclide content in the plants. An increase in the organic fertilizer component to 30% caused neither yield enhancement, nor the restriction of radionuclide accumulation in the plants.

Similar results were obtained for winter wheat, oats and barley (Lyaskovsky et al., 1995). The ^{90}Sr and ^{137}Cs amounts in wheat straw were 4–5 times higher than those in the grain. In order to limit more effectively the radionuclide uptake by plants a new variant was included in the experiments.

Table 4
^{90}Sr and ^{137}Cs contents in spring wheat plants cv Saratovskaya 29, Bq/kg

Variants	Straw		Grain		Straw		Grain	
	^{90}Sr	CA*	^{90}Sr	CA	^{137}Cs	CA	^{137}Cs	CA
Control	133.2± 0.07	1.64	25.9±1.8	0.32	170.2±12.9	0.55	51.8±4.1	0.17
CSAMF	48.1±1.80	0.59	14.8±0.7	0.18	74.0±1.9	0.24	40.7±0.8	0.13
CSAOMF (17% ML)	44.4±4.1	0.55	12.5±1.0	0.15	70.3±0.9	0.23	29.6±0.3	0.10
CSAOMF (30% ML)	50.1±3.8	0.62	15.9±0.9	0.20	82.9±4.4	0.27	32.2±0.6	0.11
CSAOMF (17% ML)+Li, Cu, Zn	45.6±0.7	0.56	7.1±0.0	0.09	66.7±2.9	0.22	22.2±2.9	0.07
$LSD_{0.05}$	7.08		1.18		8.77		2.93	
$LSD_{0.05\%}$	11.01		7.68		9.45		8.32	

*CA - coefficient of radionuclide accumulation

In this variant the microelements Li, Cu and Zn were added to organomineral fertilizer with the optimum content of humus-like substances (17% ML). According to some researchers (Gudkov, 1991) the above microelements are able to limit the uptake of radionuclides by plants. The addition of these microelements to the fertilizer caused a decrease in the ^{90}Sr and ^{137}Cs content in the straw and grain of spring wheat.

It can thus be concluded that the form of organomineral fertilizer intended for cereals could be an important factor in increasing their productivity and improving their yield quality.

Beetroot yield and ^{90}Sr and ^{137}Cs accumulation in plant tissues

The form of organomineral fertilizer (NPK ratio 1:1.5:2) intended for vegetables showed high efficiency in the experiments with beetroot plants (Table 5).

Table 5
Effect of CSAOMF on the yield of beetroot cv Bordo and on its quality

Yield and yield quality	Variants		
	Control N:P:K-1:1.5:2	CSAOMF N:P:K-1.5:1:1	CSAOMF N:P:K-1:1.5:2
Root crop mass, g*	122.5±13.9 100	121.3±16.4 99	201.5±29.8 165
Sugar content, %	13.5±0.3 100	13.9±0.5 103	12.9±0.2 96
Sugar content, g/single beet	16.5±1.0 100	16.9±0.8 102	26.0±1.3 158
NO_3^-, mg/kg fresh weight	29.2±3.6 100	24.9±0.1 85	48.6±1.4 166
Dry matter, %	21.2±0.02 100	21.3±0.06 101	20.0±0.05 96

*$LSD_{0.05}$= 19.95, $LSD_{0.05\%}$= 13.44

As compared with the control plants the root crop productivity was 64% higher, whereas both the dry matter and sugar contents in the tissues were somewhat reduced. However, on converting the sugar content to g/single beet it was found to increase by 58% as compared with the control. The stimulation of root crop growth in this variant was accompanied by the intensification of mineral element uptake, particularly that of nitrogen. It was this that led to the enrichment of root crop tissues with nitrates. However, their content was still approximately 10 times lower than the permissible concentration for beetroot – 1400 mg/kg (Opopol and Dobryanskaya, 1986). The ^{90}Sr and ^{137}Cs accumulation in beetroot was 3–10 times higher than that in cereal grains (Table 6).

These data confirm the necessity of elaborating new forms of organomineral fertilizers specially designed for growing vegetables and root crops. These plants are able to accumulate great quantities of radionuclides, heavy metals and other harmful elements in their root crops. It is curious that beetroot plants accumulate a large quantity of ^{137}Cs in their root crops and tops. This amount is 3–4 times higher than the ^{90}Sr content in these organs. Since beetroot has a large potassium requirement, it can be assumed that the ^{137}Cs is passively absorbed from the soil together with potassium and is then accumulated in the plant tissues. In spite of this the application of organomineral fertilizer makes it possible to reduce the ^{90}Sr and ^{137}Cs accumulation 2–3 times.

Table 6

Effect of CSAOMF on the ^{90}Sr and ^{137}Cs accumulation in beetroot cv Bordo, Bq per kg

Variants	Tops		Root crops		Tops		Root crops	
	^{90}Sr	CA*	^{90}Sr	CA	^{137}Cs	CA	^{137}Cs	CA
Control N:P:K-1:1.5:2	150±13	0.42	670±70	1.86	765±47	1.47	2165±117	4.16
CSAOMF N:P:K-1.5:1:1	95±11	0.26	390±36	1.08	360±24	0.69	840±55	1.62
CSAOMF N:P:K-1:1.5:2	65±6	0.18	265±29	0.74	395±25	0.76	1055±67	2.03
$LSD_{0.05}$	7.21		43.87		26.00		65.78	
$LSD_{0.05\%}$	6.98		9.93		5.13		4.86	

*CA – coefficient of radionuclide accumulation

Hence, the organomineral fertilizer intended for vegetable crops, with an NPK ratio of 1:1.5:2, may be used to significantly increase beetroot productivity, improving the yield quality and decreasing the ^{90}Sr and ^{137}Cs accumulation in the plants.

Discussion

The development of the root system is known to be under the control of genotype, though the extent to which this genetic control is manifested is determined by environmental factors (Schiefelbein and Benfey, 1991).

Among these the physico-chemical properties of the soil and the mineral nutrients in the plants play a very important role (Ustimenko et al., 1975; Marschner, 1983). Organic substances, particularly humus, improve the soil

structure and stimulate root growth, thus intensifying root system formation (Diakonova and Maksimova, 1986).

The application of organomineral fertilizer in spring wheat production caused a strengthening of the root system, an increase in the root:shoot ratio (Table 1) and a change in the plant architecture (Table 2). These differences in plant morphological structure resulted from changes in source-sink relations between the roots and shoots under the influence of this fertilizer. The root system, receiving photoassimilates from the aboveground parts, used their carbon structures as building materials and energy sources in the processes of metabolism and growth, as well as in the synthesis of phytohormones (Torrey, 1976; Feldman, 1984; Ronzhina and Mokronosov, 1994) and amino and organic acids (Klimashevskaya, 1986; Izmailov, 1986). By transporting these compounds into the aboveground parts, together with mineral elements and growth substances absorbed from the granules of organomineral fertilizers and the soil, the root system acted as a donor for a number of vital metabolites which, in the course of shoot growth and metabolism, regulate the formation of shoot morphological structure. Thus, by stimulating the growth of the root system the components of organomineral fertilizers enhance the ability of the roots to attract assimilates from the aboveground parts and to use them in metabolic processes. This in turn strengthens the donor function of the root system.

By retarding upward shoot growth the physiologically active substances transported from the roots into the aboveground parts of the plant cause changes in the distribution of photoassimilates in the plant organism. Part of these assimilates are transported to the roots and used in the processes of metabolism. Another part is retained in the shoot tissues as a result of both the increase in phytohormone content and the inhibition of upward stem growth (Lyaskovsky, 1991a). The enrichment of the tissues with metabolites induces the biosynthesis of stem skeleton compounds (cellulose, hemicelluloses, pectic substances and lignin) (Lyaskovsky, 1989a,b; 1991a,b). It is this that reinforces the development of the stem anatomical structure and the mechanical strength of the straw (Prusakova, 1985; Lyaskovsky, 1991a). Therefore, the regulation of the source-sink relations between the plant organs by means of the composition of the organomineral fertilizer supplies the necessary conditions for the development of a compact plant architecture, which, as proved in the literature (Prusakova, 1985; Lyaskovky, 1991a), contributes to resistance to both lodging and unfavourable environmental factors (drought and low temperature).

By strengthening root system formation and delaying its aging and dying off in the reproductive stages of plant development, organomineral fertilizer favours more intensive nitrogen accumulation in experimental plant tissues (Fig. 3). Nitrogen entering the plant in the form of NO_3^- is accumulated mainly within young plant tissues (Izmailov, 1986). This induces nitrate reductase activity (Li and Oaks, 1993; Imsande and Touraine, 1994; Raab and Terry, 1995), which results in nitrogen reduction, assimilation and deposition in the tissues of vegetative mass in the form of nitrogenous substances. Non-reduced nitrogen is reserved in non-metabolic compartments of the cell in the form of NO_3^- (Ovcharenko et al., 1990).

The great ability of the growing flag-leaf at the heading stage to attract nutrients caused maximum nitrogen level and nitrate reductase activity in its tissues (Figs 2 and 5). No enzyme activity was observed in the stem, especially at the bottom (Fig. 4), which contains less nitrogen. The decline in nitrogen uptake in plants during grain ripening, and the reutilization of nitrogen-containing substances in the organs investigated was accompanied by a decrease in the nitrate reductase activity of the flag-leaf (Fig. 5).

At the stages of grain filling and ripening, nitrogen is absorbed by the roots and transported in the form of NO_3^- mainly to the ear, avoiding other organs, especially the aging flag-leaf (Izmailov, 1986). Literary data (Shargaeva et al., 1985; Li and Oaks, 1993; Raab and Terry, 1995) indicate that the nitrogen reserved in the growing roots induces nitrate reductase activity in their tissues. The increase in enzyme activity in the roots at these stages of development evidently compensates in part for the loss of the flag-leaf contribution to nitrogen assimilation because of the decreasing nitrate reductase activity in its tissues. This exchange of enzyme activity distribution between the flag-leaf and the roots is known to be characteristic of many crops and was observed by other researchers (Shargaeva et al., 1985; Ovcharenko et al., 1990). Some authors found a relationship between nitrate reductase activity and the protein content in the grain (Croy and Hageman, 1970; Pavlov, 1984). The wheat ancestors *Aegilops speltoides* Tausch and *Aegilops squarrosa* L., for instance, which possess well-developed root systems and high nitrate reductase activity, accumulated more protein in the grain than modern wheat varieties (Shargaeva et al., 1985). Hence, by stimulating the growth of the root system and increasing nitrate reductase activity organomineral fertilizer favours the intensification of nitrogen uptake by the roots, its assimilation in the tissues and the accumulation of nitrous substances and mineral nitrogen in the vegetative mass. Mineral nitrogen is reserved in the non-metabolic compartment of the cell. This form of nitrogen is reutilized in the period of grain formation and ripening and, alongside the nitrogen absorbed by the roots, is known to be a source for protein synthesis (Pavlov, 1984; Chanh, 1991).

The reduction in photosynthesis intensity during the period of grain filling and ripening causes various organic compounds deposited in the vegetative mass to be included in the metabolism (Lyaskovsky, 1989a,b; 1991a,b; Kumakov et al., 1991). This results in the appearance of a large number of active intermediates of an organic and inorganic nature. The carbon structures required for assimilation are synthesized in deficient amounts due to the reduction in the intensity of photosynthesis. There is thus a great need for these carbon structures. In addition, the root system continues to function at different intensities depending on the variety and the cultivation conditions, thus supplying the aboveground plant parts with nitrogen and other mineral elements, whose assimilation also requires carbon structures. An especially critical situation is observed in the ear, to which nitrogen is transported directly from the root system in this period. This is why the intensity and duration of the root system activity play a very important role in protein accumulation within the

grain. On the basis of this, modern intensive wheat cultivation technologies include nitrogen top dressing at the final stages of plant development (Pavlov, 1984).

Thus, organomineral fertilizer increases the duration of the vital activity of the root system and other organs, as well as strengthening their functional activities, thus contributing to nitrogen uptake at the final stages of plant development and to the resultant increase in the grain yield, the protein content, the gluten content and the total and essential amino acids (Table 3).

The inclusion in the metabolism of polysaccharides from the vegetative mass and particularly from the stem, compensates for the lack of carbon structures observed in the period of reproductive development. The stem polysaccharides perform the role of skeleton formation and thus determine the mechanical strength of the straw. Being hydrolysed they play the function of storage substances (Lyaskovsky, 1989a, 1991a; Kumakov et al., 1991). It was shown earlier with the use of $^{14}CO_2$ that the products of stem polysaccharide hydrolysis were incorporated in the processes of glycolysis and gluconeogenesis and appeared to be the main source of carbon structures in this period (Lyaskovsky, 1989a,b; 1991a,b). They are obviously used for three main purposes: 1) for the assimilation of nitrogen from reutilized nitrogenous substances from the plant vegetative mass; 2) for the assimilation of mineral nitrogen released from storage compartments; 3) for the assimilation of nitrogen absorbed by the roots from the soil.

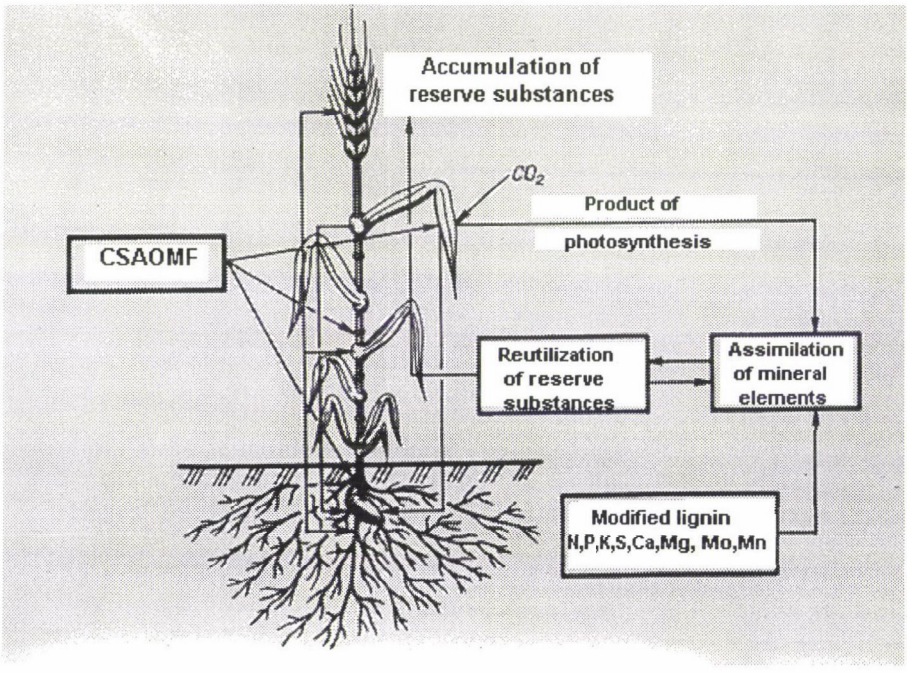


Fig. 6. Control of morphostructure formation and increase in plant productivity under the influence of CSAOMF components. Explanation in the text

The excess of metabolites resulting from hydrolytic processes is deposited in the stem in the form of oligosaccharides, which act as temporary transitory storage compounds (Lyaskovsky, 1989a; 1991b). Their intensive formation in cereals during high tension physiological-biochemical processes or at the stages which precede these processes (flowering, grain filling and ripening) points to the important role of these substances in metabolic processes (Babenko, 1971). The dependence of nitrogen transport in plants on their carbohydrate contents (Barneix et al., 1992) leads to the assumption that the rapid transformation of stem oligosaccharides into sucrose and its outflow to the ear, observed in the period of grain filling (Lyaskovsky, 1991b), reinforces both nitrogen reutilization and transport as well as its assimilation in the kernels. This idea is supported by the fact that the depletion of the tissues in carbohydrates, caused by artificial or natural plant shading when plants lodge under field conditions, gives rise to an inhibition of nitrogen reutilization and transport and to a decline in its accumulation in the kernels (Guitman et al., 1991).

Thus, the reutilization of polysaccharides from the vegetative mass is very important not only for grain filling and ripening, but for the general metabolism too. Obviously these compounds participate in the biosynthesis of carbon structures (skeleton), the synthesis of which is delayed during ripening. However, the requirements for these structures are very great as the result of the reutilization of the organic substances and minerals reserved in non-metabolic compartments of the cell.

The regulation of wheat plant morphostructure and the increase in the functional activity of plant organs using complex organomineral fertilizers can be described in the following manner (Fig. 6). By improving the soil structure and raising the content of minerals and humus, complex organomineral fertilizer intensifies both the formation and growth of the root system and reinforces the source-sink relationships between plant organs. By attracting assimilates from the aboveground plant parts, the root system includes their carbon structures in the biosynthesis of phytohormones and vital metabolites. By transporting these substances to the shoots, the root system causes the directed formation of the plant architecture and strengthens the functional activity of the plant organs. As a result of this, the roots absorb biologically important elements more effectively and discriminate against inert ones (Lyaskovsky et al., 1995). This discrimination may be conditioned by the stimulation of the activity of special Ca^{2+}- and K^{+}-ATPases, which are located in the plasmolemma of root hair cells. They selectively transport Ca^{2+} and K^{+} ions and discriminate against $^{90}Sr^{2+}$ and $^{137}Cs^{+}$. The absorption of biologically important elements by the roots causes their accumulation in plant tissues in the form of albuminous and mineral compounds. In the period of grain filling and ripening they are reutilized and serve as a source of metabolites for the biosynthesis of grain storage compounds. Modified hydrolytic lignin, as a humus-like substance, is a component in organomineral fertilizer and increases the humus content of the soil, thus favouring the formation of complex high-molecular substances, which

fix minerals in the soil and limit their migration into the water and atmosphere. Thus, the creation and application of new forms of organomineral fertilizers may be an important factor for the development of sustainable plant productivity and biosphere, as well as activating internal plant reserves, which are necessary for the realization of biological potential.

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EFFECT OF NITROGEN, NITRIFICATION INHIBITOR DCD AND DURATION OF VARIETY ON PRODUCTIVITY AND NITROGEN UPTAKE OF RICE

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A field experiment conducted for 2 years at the Indian Agricultural Research Institute, New Delhi showed that a very short duration variety, Pusa Jaldi Dhan-1, could produce a grain yield of only 1 t/ha as against 4–5 t/ha produced by the medium duration variety Pusa Basmati-1. Pusa Jaldi Dhan-1 responded only up to 60 kg/ha, while Pusa Basmati-1 responded up to 120 kg N/ha. Nitrogen uptake by Pusa Jaldi Dhan-1 was only one third to one half of that by Pusa Basmati-1. The apparent recovery of applied N was also much lower in Pusa Jaldi Dhan-1. No advantage was gained by treating urea with the nitrification inhibitor DCD.

Key words: rice, nitrogen, nitrification inhibitor, short/medium duration

Introduction

Rice is the staple food of millions of poor people in Asia, where 90% of the world's rice is grown and eaten (IRRI, 1989). India produces about one-fifth of the world's rice and it is estimated that by the year 2000 AD, India will need about 94 million tonnes of rice (Siddiqi, 1996) as against an estimated production of 80.5 million tonnes in 1995–96 (FAO, 1997). There is very little scope for increasing the area under rice in India and much of the increase in rice production has to come from an increase in productivity (Biswas et al., 1991). Nitrogen is a major component of the agronomic package for increasing rice productivity, but its use efficiency is very low in rice; only 25–40% of applied N is taken up by the rice crop (Prasad and De Datta, 1979). Nitrification inhibitors are reported to increase the efficiency of N applied to rice (Prasad and Power, 1995). Again, of late some very short duration rice varieties have become available for use as a catch crop in multiple cropping systems or for introduction in new areas, such as *diara* lands after the floods recede. No information on productivity or response to N is available for new, very short duration varieties. Therefore a study was taken up at the Indian Agricultural Research Institute to determine the productivity and the response to N and nitrification inhibitor DCD of a very short duration variety, Pusa Jaldi Dhan-1, vis-a-vis a traditional medium duration variety, Pusa Basmati-1.

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Materials and methods

The field experiments were conducted during the *kharif* seasons (July–November) of the crop years (July–June) 1990–91 and 1991–92. A split plot design with varieties (Pusa Jaldi Dhan-1 and Pusa Basmati-1) in the main plot and combinations of nitrogen levels (60, 120 kg N ha^{-1}) and DCD treatments (with and without DCD and a no-nitrogen control) in the sub-plots was adopted. There were three replications. The soil of the experimental field was a sandy clay loam alluvium of pH 8.0 with 0.75% organic C and medium quantities of available P and K.

Materials

Pusa Jaldi Dhan-1, developed at the Indian Agricultural Research Institute, New Delhi from the cross Gora M/MW-10M/N-22M (M is an induced mutant; N-22 refers to Nagina 22), is a medium tall variety with aggressive early growth and a crop duration of 70–75 days. It is specially suitable as a catch crop in intensive multiple cropping systems or for post-flood sowing on flood-prone areas (*diara* lands).

Pusa Basmati-1, developed at the Indian Agricultural Research Institute, New Delhi, is a cross between Pusa 167 and Karnal Local Basmati. It is a semi-dwarf variety of 135–140 days' duration and has long slender aromatic grains. It is suited for irrigated rice culture in northwestern India.

Dicyandiamide (DCD) is a nitrification inhibitor and was obtained from SKW Trostberg, Germany. It is a white amorphous material and contains 66% N. Urea was coated with DCD using linseed oil (2 ml kg^{-1}) as a sticker in a rotating drum. The required quantity of DCD (77 g kg^{-1} urea) was then added and the contents were thoroughly mixed. This product had 10% N as DCD.

Methodology

The experimental field was disk-ploughed twice, puddled with a country plough in standing water and finally levelled. Before puddling 20 kg ha^{-1} each of P (as ordinary superphosphate) and K (as muriate of potash) and 5 kg ha^{-1} of $ZnSO_4$ was applied as basal fertilizer. Two to three 25-day-old seedlings were transplanted per hill (at 20 cm × 10 cm spacing). Half the N dose was applied as per treatment 10 days after transplanting (DAT) when the plants were established. The rest was applied 20 days after the application of the first half.

The crop was irrigated as and when necessary (8 irrigations in 1990 and 10 irrigations in 1991). Data on different yield attributes and on the grain and straw yields were recorded at harvest. Samples of grain and straw were taken from each plot and analysed for total Kjeldahl N (Prasad, 1997). Nitrogen uptake and apparent N recovery were then computed (Prasad, 1996).

Results and discussion

Yield attributes

Of the 3 factors studied, only the varieties differed significantly in respect of yield attributes in both the years, while N levels and DCD had no significant influence (Table 1). Of the 2 varieties studied, Pusa Basmati-1 had significantly longer and heavier panicles and a larger total number of spikelets, while Pusa Jaldi Dhan-1 had a significantly higher percentage of filled spikelets and test weight. Srivastava (1981) reported that due to the overlapping of the vegetative and reproductive growth stages in short duration varieties, the panicles tend to be shorter and lighter in short duration varieties and this is responsible for their lower yields. As regards the percentage filled spikelets and test weight Tanaka et al. (1964) and Reddy and Prasad (1977) pointed out that as the number of total

Table 1
Yield attributes of rice as influenced by variety, nitrogen and DCD (averaged over 2 years)

Factor	Panicle length (cm)	Panicle weight (g)	Total spikelets (No.)	Filled spikelets (%)	Test weight (g)
Variety					
Pusa Jaldi Dhan-1	19.0	1.1	60.1	86.1	22.2
Pusa Basmati-1	26.3	2.4	152.4	79.0	19.7
$LSD_{5\%}$	0.9	0.2	10.2	3.7	0.4
Nitrogen (kg/ha)					
0	21.6	1.5	92.0	79.7	20.6
60	22.3	1.7	104.7	83.1	21.0
120	23.0	1.8	110.7	83.0	21.1
$LSD_{5\%}$	NS	NS	NS	NS	NS
DCD					
Without	21.8	1.7	110.2	82.6	21.0
With	22.0	1.7	107.1	83.3	20.9
$LSD_{5\%}$	NS	NS	NS	NS	NS

NS – non-significant

spikelets increased, as in the case of Pusa Basmati-1, the percentage filled grains and test weight tended to decrease due to the reduced accumulation of starch in the spikelets and inter-spikelet competition for light and nutrients.

Grain and straw yield

Grain and straw yield were significantly influenced by variety and nitrogen but not by DCD (Table 2). Furthermore, the variety × nitrogen interaction was significant in both the years of study. Data are therefore presented for both the years separately. In 1990, Pusa Jaldi Dhan-1 produced 0.9 t ha^{-1} grain without N and there was no significant response to N, while Pusa Basmati-1 produced 3.6 t ha^{-1} grain and there was a significant increase of 0.5 t ha^{-1} with the application of 60 kg N ha^{-1}. In 1990 there was no additional significant increase in the grain yield of Pusa Basmati-1 when the level of N was raised to 120 kg N ha^{-1}. In 1991 Pusa Jaldi Dhan-1 produced only 0.3 t ha^{-1} grain, while Pusa Basmati-1 produced 3.5 t ha^{-1} grain in no nitrogen plots and in both the varieties a significant increase was only recorded when 120 kg N ha^{-1} was applied. The lower yield of Pusa Jaldi Dhan-1 in 1991 was due to the lower number of rainfall weeks in 1991 (50%) as compared to 1990 (70%). Maximum temperatures were also higher in July 1990 (39–42°C) as against 32–38°C in 1991. Sharma et al. (1990) also reported that a medium duration rice variety, Pusa 169, responded to N application up to 120 kg N/ha, while the response of a short duration rice variety, IR-50, was restricted only up to 60 kg N/ha.

As regards the straw, the 'Variety × N' interaction was significant in 1991 (Table 3) and Pusa Basmati-1 produced significantly more straw than Pusa Jaldi Dhan-1 at all levels of N. The difference between 0 and 60 kg N/ha was not significant in either of the varieties, but the difference between 60 and 120 kg N/ha was significant, indicating that only when higher levels of N were applied, there was an increase in the straw yield.

Table 2
Grain and straw yield (t/ha) and harvest index (HI, %) of rice varieties as influenced by N levels

Variety	1990			1991		
(kg N/ha)	Grain	Straw	HI	Grain	Straw	HI
Pusa Jaldi Dhan-1						
0	0.9	3.6	19.5	0.3	1.6	13.9
60	1.1	3.9	21.7	0.9	2.8	23.9
120	1.1	4.1	21.9	1.3	4.3	23.3
Pusa Basmati-1						
0	3.6	11.4	24.0	3.5	8.4	29.7
60	4.1	12.4	24.9	3.5	9.8	32.9
120	4.2	13.4	24.4	5.4	11.6	32.0
$LSD_{5\%}$						
Between levels						
0 vs 60	0.32	NS	NS	0.64	NS	7.0
60 vs 120	NS	NS	NS	0.45	1.02	NS
Between varieties						
at 0	0.37	NS	NS	0.73	1.67	8.1
at 60	0.26	NS	NS	0.52	1.18	5.7
at 120	0.18	NS	NS	0.37	0.84	4.0

NS – non significant

The treatment of urea with DCD had no significant effect on the rice yield in the present study.

In respect of the harvest index (HI) the 'variety × N' interaction was significant only in 1991. A significant increase in HI was obtained in Pusa Jaldi Dhan-1 when the first increment of 60 kg N/ha was applied, there being no further significant increase when the level of N was raised to 120 kg N/ha. The difference between levels of N was not significant in Pusa Basmati-1. The harvest index was higher in Pusa Basmati-1 than in Pusa Jaldi Dhan-1 at all levels of N.

Nitrogen uptake

Data on nitrogen uptake are given in Table 3. As would be expected from the data on grain and straw yield, N uptake in Pusa Basmati was significantly greater (nearly 3 times) than in Pusa Jaldi Dhan-1 in both years of the study. In the case of Pusa Jaldi Dhan-1, a significant increase in N uptake in the grain was obtained in both the years with the first increment of 60 kg N/ha, but there was no additional significant increase in N uptake when the level of N was increased to 120 kg N/ha. On the other hand a significant increase in N uptake by the straw of Pusa Jaldi Dhan-1 was only obtained in both the years when 120 kg/ha was applied. In the case of Pusa Basmati-1, a continuous significant increase in N uptake by the grain was recorded up to 120 kg N/ha. However, as regards the straw, the results were similar to those found for Pusa Jaldi Dhan-1. This shows that the first increment of N was utilized for grain production, and only when additional N was available to the crop was it stored in the straw.

Table 3
Nitrogen uptake (kg/ha) and apparent N recovery by rice varieties as influenced by N levels

Variety	1990			Apparent N	1991			Apparent N
(kg N/ha)	Grain	Straw	Total *	recovery*(%)	Grain	Straw	Total*	recovery*(%)
Pusa Jaldi Dhan-1								
0	11.0	15.8	26.8	–	11.0	8.3	19.3	–
60	15.7	18.6	34.3	12.5	15.7	17.2	32.9	22.6
120	16.6	21.5	38.1	9.4	16.6	36.6	53.9	28.8
Pusa Basmati-1								
0	38.8	50.3	89.1	–	28.7	33.8	62.5	–
60	48.6	58.2	106.8	29.5	34.6	44.1	78.7	27.0
120	52.8	69.7	122.5	27.8	58.9	52.9	111.8	41.0
$LSD_{5\%}$								
Between levels								
0 vs 60	5.3	NS			4.9	NS		
60 vs 120	3.8	9.5			3.5	7.3		
Between varieties								
at 0	6.1	15.6			5.8	12.0		
at 60	4.3	10.9			4.1	8.5		
at 120	3.1	7.8			2.9	6.0		

* Data not subjected to statistical analysis; NS – non significant

Total grain + straw N uptake by Pusa Basmati-1 was twice to 3 times that of Pusa Jaldi Dhan-1 and the apparent recovery of N was also much lower in Pusa Jaldi Dhan-1. Thus, Pusa Jaldi Dhan-1 was not an efficient user of fertilizer nitrogen.

No advantage was gained by treating urea with DCD in the present study. DCD is reported to decompose very fast at temperatures above 25°C (Vilsmeier, 1981) and this could be one reason why it was not effective in increasing the efficiency of N applied to rice; temperatures during rice growth at Delhi are 30±5°C. After carrying out field experiments on flooded rice Simpson et al. (1985) also reported that DCD addition to urea had no significant effect on the fertilizer recovery by rice. Our earlier studies at IARI (Sudhakara and Prasad, 1986) also indicated no advantage of DCD in upland rice. DCD is thus not an effective nitrification inhibitor for rice.

The results of the present study indicate that very short duration varieties of rice such as Pusa Jaldi Dhan-1 are very poor yielders and inefficient users of fertilizer N and should be grown only under specific conditions, such as when the moisture is receding on flood-prone areas popularly known as *diara* lands. In such areas irrigation water is not available and the crop can be grown only on stored moisture and therefore has to be a very short duration one. Because of their poor yielding capacity, very short duration rice varieties such as Pusa Jaldi Dhan-1 will not fit in multiple cropping systems even as a catch crop.

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STUDIES ON THE OPTIMISATION OF SEEDLING AGE, NITROGEN DOSE AND SPLIT APPLICATION FOR LOWLAND RICE ESTABLISHED BY THE SEEDLING THROWING METHOD

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A field experiment was conducted during the 1995–1996 wet and dry seasons to optimise the seedling age, nitrogen dose (N) and split applicataion for the seedling throwing method of planting lowland rice. The seedling throwing method was compared with the line and random planting methods. In the dry season the yield (6.35 t/ha) achieved with the seedling throwing method was significantly greater than that in the line and random planting methods (6.15 and 5.71 t ha^{-1}, respectively). The grain yield of the line planted crop was higher (5.84 t/ha) in the wet season, but was at par with the yield of the seedling throwing method (5.61 t/ha). During both seasons random planting (farmer's method) led to markedly lower yields (5.22 t ha^{-1} and 5.71 t ha^{-1} in the wet and dry seasons, respectively). Although the line planting method enhanced the yield to 5.84 t ha^{-1} and 6.15 t ha^{-1} during the wet and dry seasons, respectively, the seedling throwing method is nevertheless more advantageous because of the lower labour requirements (44.5% labour saving compared to line planting over the seasons) and the relative ease with which the seedlings can be thrown. The advantage of the seedling throwing method resulted in a higher benefit:cost (B:C) ratio (2.85 and 3.14 for the wet and dry seasons) compared to the line planting method (2.79 in the wet and 2.95 in the dry season). Studies on the evaluation of component technologies to enhance the yield of seedling-thrown rice revealed that in the wet season, irrespective of the N dose applied, the use of older seedlings (30 or 40 days old) with four split N applications maximised the yield to 6.03 t ha^{-1} compared to that of the line-planted crop (5.84 t ha^{-1}). In the dry season, the throwing of 35-day-old seedlings with the application of 180 kg N ha^{-1} in five split applications enhanced the yield to 7.32 t ha^{-1}, which was significantly more than that of line planting (6.15 t ha^{-1}).

Key words: rice planting methods, seedling throwing, seedling age, nitrogen dose, split application

Introduction

As rice is a semi-aquatic plant, the transplanting method of establishment is most favourable and in general is believed to give a more stable grain yield than direct seeding methods (Biswas et al., 1991). Although the direct seeding method is cost-effective, the grain yield is generally low due to poor and non-synchronised tillering and severe weed infestation. Transplanting (line planting) is seldom practised by the farmers of Tamil Nadu, obviously due to socio-economic considerations. The rice is transplanted randomly by women, who

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transplant a far lower number of hills/unit area, resulting in low yields (Gill et al., 1989). The need for a satisfactory alternative to the transplanting of rice, in the context of the scarcity and increasing cost of labour and the desire to reduce the drudgery of the women, has been very much felt and attempts were made in this direction. Though mechanical rice transplanting has been developed, it is not suitable for small, fragmented holdings or for the socio-economic conditions of rice farmers in India. In South East Asian countries, a method of rice crop establishment by simply throwing the rice seedlings into the puddle has been developed to achieve better management and yield in rice cultivation (Matsushima, 1979). In Kerala, Varughese et al. (1993) also studied the seedling throwing method of rice planting in the context of the scarcity of human labour and the higher costs and drudgery involved in transplanting.

Rice is highly sensitive to diverse physiological traits, and seedling age at transplanting plays a crucial role in obtaining a uniform crop stand and thus achieving the potential yield. Though a linear decline in yield was obtained when older seedlings were transplanted, Matsushima (1979) recommended older seedlings for the seedling throwing method of rice planting. Crop yield is influenced, often decisively, by the extent to which the plant requirement for N can be met. This varies greatly from crop to crop and soil to soil and from one climate to another. The data on N utilisation by rice in India reveal that the recovery of applied N ranged from 19.1 to 39.8% (Meelu, 1980). The split application of N is perhaps the simplest agronomic solution for improving the use efficiency of N, since the N demand is not the same throughout the plant growth period (Manjappa et al., 1994). Although a lot of research has been done on the traditional transplanting method, there is a paucity of information about seedling age, dose and split application of N for the seedling throwing method.

The objectives of this study were to evaluate the suitability and success of the seedling throwing method of rice planting and to optimise the seedling age, N dose and split application for this planting method in order to obtain a yield comparable with that of the line and random planting methods, while involving lower labour requirements and greater ease of operation.

Materials and methods

The experiments were conducted on the wetlands of Tamil Nadu Agricultural University, Coimbatore during the wet season (October 1995 to February 1996) and the dry season (June to September 1996). The soil texture was clay loam classified as Typic Haplustalf, with a pH of 8.0. The soil was low in available N (198.5 kg ha^{-1}), medium in phosphorus (18.6 kg ha^{-1}) and high in potassium (557 kg ha^{-1}).

The experiment was laid out in a factorial randomised block design with three replications. The twelve treatment combinations involved in the seedling throwing method in the wet season included three seedling ages (20, 30 and 40 days old), two N doses (150 and 187.5 kg ha^{-1}) and two types of split applications (three and four). In the dry season, two seedling ages (25 and 35 days old), three N doses (120, 150 and 180 kg ha^{-1}) and two types of split applications (four and five) were studied. The new method of rice planting, involving a seedling-thrown crop under the

above twelve treatment combinations, was compared with line- and random-planted crops that were grown with the recommended production practices.

The rice varieties ADT38, of medium duration, and ADT36, of short duration, were utilised during the wet and dry seasons, respectively. The field was puddled and uniformly levelled. In the line planting method, 30-day-old seedlings from a wet nursery were transplanted at a spacing of 20 × 10 cm in the wet season and 25-day-old seedlings at a spacing of 15 × 10 cm in the dry season. Random planting was done using 30- and 25-day-old seedlings (in the wet and dry seasons, respectively) from a wet nursery without adopting any definite plant geometry and density, as done by farmers. A uniform dose of P_2O_5 and K_2O (each of 50 kg and 38 kg ha^{-1} during the wet and dry seasons, respectively) was applied in the form of superphosphate (16% P_2O_5) and muriate of potash (60% K_2O), while N as urea (46% N) was applied as per treatment. Nitrogen was applied in three splits: 50% basal, 25% each at active tillering (AT) and panicle initiation (PI); four splits: 25% each at 10 days after seedling throwing (DAST), AT, PI and flowering (F), and five splits: 10 DAST, AT, PI, F and 10 DAF as per treatment. Full doses of P_2O_5 and $ZnSO_4$ (25 kg/ha) were applied at the last puddling and K_2O was applied in equal splits along with N.

Seedlings from a wet nursery, of different ages as required by the treatment, were used in the seedling throwing method. Two or three seedlings were separated as in normal transplanting and were thrown at random into the respective plots by hand from a standing position without using force. In the seedling-thrown plots, 25% more seedlings were used than in the line planting method, calculated according to the plant spacing adopted in the respective seasons. The labour requirements for each method of planting were recorded and given in woman days ha^{-1}. The growth parameters and yield components were recorded by adopting standard procedures. The grain yield was recorded from a net plot area of 6 m^2 and expressed in t ha^{-1} at 14% moisture.

Results and discussion

Effect of planting methods on growth, yield parameters and yield

The response of individual growth and yield parameters varied significantly with the different planting methods (Table 1). The leaf area index was comparable in the line planting (5.70) and seedling throwing methods (5.53) in the wet season. However, in the dry season the seedling throwing method gave significantly higher LAI values (5.26) compared to that of the other planting methods. The development of profuse lateral roots by the seedling-thrown crop might have favoured better nutrient uptake and tiller production at an early stage (Matsushima, 1979). Although the line planting method resulted in more panicles m^{-2} (488) compared to seedling throwing (471) in the wet season, the reverse was true in the dry season (483 and 459 panicles m^{-2} for the seedling throwing and line planting methods, respectively). Varughese et al. (1993) also recorded a higher number of panicles m^{-2} with the seedling throwing method compared to transplanting. The increased number of panicles m^{-2} with the seedling throwing method might be due to the greater number of tillers at the flowering stage. Although the rice planting method had no significant influence on the number of filled grains in the wet season, the seedling throwing method led to a significantly larger number of filled grains per panicle (88.8) and was on a par with the line planted crop (86.0) in the dry

season. Higher spikelet sterility was observed with the seedling throwing method (16.7 and 19% in the wet and dry seasons, respectively) compared to the line planting and random planting methods. Higher spikelet sterility in the seedling throwing method of planting was also reported by Shekinah (1996). It is evident that for the yield parameters studied the seedling throwing method is superior to the random planting method. The seedling throwing method produced grain yields at par with the line planting method in the wet season, while in the dry season the seedling throwing method registered the highest grain yield (6.35 t ha^{-1}, compared to 6.15 t ha^{-1}) for line planting (Table 1). The random planting method gave poor yields (5.22 t ha^{-1} in the wet and 5.71 t ha^{-1} in the dry season). Kenchiah et al. (1996) also recorded the highest grain yield with line planting, but the seedling throwing method gave yields comparable with that of the line-planted crop. This might be due to the better growth and yield attributes recorded in the seedling throwing and line planting methods compared to random planting.

Table 1

Effect of planting methods on growth, grain yield and economic indices of wet and dry season rice

Characters	Wet season				Dry season			
	Seedling throwing	Line planting	Random planting	$CD_{5\%}$	Seedling throwing	Line planting	Random planting	$CD_{5\%}$
Leaf area index	5.53	5.70	5.18	0.24	5.26	4.95	4.50	0.28
Panicles m^{-2}	471	485	450	13	483	459	417	14
Filled grains $panicles^{-1}$	88.2	92.5	90.8	NS	88.8	86.0	84.1	3.5
Unfilled grain panicle (%)	16.7	12.4	16.1	1.90	19.0	16.1	15.8	2.01
Grain yield (t ha^{-1})	5.61	5.84	5.22	0.37	6.35	6.15	5.71	0.15
Net income (US $)	491	502	425	–	582	516	439	–
B:C ratio	2.85	2.79	2.57	–	3.14	2.95	2.84	–
Planting labour requirement (woman days ha^{-1})	30	56	46	–	31	54	43	–
Labour saving (%) over line planting	46.4	–	17.9	–	42.6	–	20.4	–

* NS – Non-significant

Effect of component techniques on the growth and yield of seedling-thrown rice

The age of the seedlings had a marked influence on LAI and other yield components of rice. In the wet season, 30- and 40-day-old seedlings gave higher and more or less the same LAI (5.78 and 5.72, respectively) compared to that with 20-day-old seedlings. In the dry season older seedlings (35 days old) also resulted in markedly higher LAI (5.60) than younger seedlings of 25 days old (4.93). Although graded N levels did not influence the LAI in the wet season crop, the application of 180 kg N ha^{-1} maximised the LAI (5.89) compared to that of lower doses (5.23 and 4.69 with 150 and 120 kg N/ha, respectively) during the dry season (Table 2).

Table 2
Effect of treatments on growth and yield components of rice established by the seedling throwing method

Treatments		Wet season				Dry season			
Wet season	Dry season	LAI	$^{+}P/m^2$	$^{++}FG/P$	$^{+++}UG/P$	LAI	$^{+}P/m^2$	$^{++}FG/P$	$^{+++}UG/P$
Seedling age (days)									
20	–	5.08	439	83.0	22.1	–	–	–	–
30	25	5.78	487	91.5	14.1	4.93	456	82.8	20.2
40	35	5.72	486	90.3	13.9	5.60	511	94.8	17.8
$CD_{5\%}$		0.17	9	3.2	1.30	0.15	8	2.0	1.10
N dose (kg ha^{-1})									
150.0	120	5.50	469	86.9	16.2	4.69	437	78.2	21.3
187.5	150	5.55	472	89.6	17.2	5.23	482	88.6	17.7
–	180	–	–	–	–	5.89	531	99.5	18.0
$CD_{5\%}$		NS	NS	NS	1.10	0.19	9	2.4	1.40
N splits									
3	4	5.35	459	85.9	17.7	5.10	471	85.6	20.2
4	5	5.70	483	90.5	15.7	5.40	496	92.0	17.8
$CD_{5\%}$		0.14	7	2.0	1.10	0.15	8	2.0	1.10

*NS – Non-significant; $^{+}$Panicles m^{-2}; $^{++}$Filled grains panicle^{-1}; $^{+++}$Unfilled grains panicle^{-1} (%)

Irrespective of the season, an increase in the number of N application splits invariably increased the LAI of the crop. The use of 30- or 40 day-old seedlings led to a substantially greater number of panicles m^{-2} (486) and filled grains panicle^{-1} (90.4) compared to that of 20-day-old seedlings (439 and 83, respectively) in the wet season. Similarly, in the dry season older seedlings (35 days old) also gave a larger number of panicles m^{-2} (511) and filled grains panicle^{-1} (94.8) compared to that of 25-day-old seedlings (456 and 82.8, respectively). Although these yield components were not influenced by N levels in the wet season, a substantial increase in the tiller production and filled grains/panicle was observed with every increment of N from 120 to 180 kg ha^{-1}, the maximum being achieved with 180 kg N/ha in the dry season. Regarding the split application of N, four splits in the wet season and five splits in the dry season gave higher numbers of panicles m^{-2} (483 and 496, respectively) compared to three (459) and four (471) splits.

The performance of 20-day-old seedlings was not encouraging, as there was a 15.9% yield reduction compared to older seedlings of 30 to 40 days in the wet season. In the dry season older seedlings also performed better than younger seedlings. The increased yield with the use of older seedlings might be due to the cumulative effect of increased growth and yield attributes. Shekinah (1996) also opined that the broadcasting of older seedlings (35 days) resulted in higher grain yield (13.7% and 7.5% during the wet and dry seasons, respectively). Padalia (1981) also stated that the restricted nutrient uptake of 20-day-old seedlings might have led to the higher sterility percentage and low yield (Table 3).

Table 3
Effect of treatments on grain yield and economic indices of wet and dry season rice established by the seedling throwing method

Treatments		Wet season			Dry season		
Wet season	Dry season	Grain yield ($t\ ha^{-1}$)	Net income (US $)	B:C ratio	Grain yield ($t\ ha^{-1}$)	Net income (US $)	B:C ratio
Seedling age (days)							
20	–	4.98	411	2.55	–	–	–
30	25	5.95	534	3.00	6.10	545	3.11
40	35	5.90	528	3.00	6.62	618	3.28
$CD_{5\%}$		0.25	–	–	0.08	–	–
N dose kg ha^{-1}							
150	120	5.55	486	2.85	5.88	526	2.99
187.5	150	5.67	495	2.85	6.33	581	3.22
–	180	–	–	–	6.82	638	3.29
$CD_{5\%}$		NS	–	–	0.10	–	–
N splits							
3	4	5.46	472	2.77	6.22	566	3.09
4	5	5.76	511	2.93	6.47	598	3.20
$CD_{5\%}$		0.21	–	–	0.08	–	–

* NS – Non-significant

The two levels of N tested did not markedly influence the grain yield in the wet season. A substantial influence of N on the grain yield was observed in the dry season. An increment of 30 kg N ha^{-1} compared with the recommended dose (120 kg N/ha) increased the yield by 7%. The application of N in four splits in the wet season and five splits in the dry season increased the grain yield by 5.3% and 3.6%, respectively, compared to that obtained with three and four splits in the respective seasons (Table 3). This might have increased the nutrient availability and uptake by the crop, resulting in a greater number of filled grains and yields. Sivasamy (1994) also reported that the application of N in a alrger number of splits ensured a steady supply of N over a longer period and hence resulted in higher biological and economic yield.

Labour requirements and economic aspects of the seedling throwing method of rice cultivation

The seedling throwing method of planting, being less labour-intensive and involving less drudgery, required only 55.5% and 68.5% of the planting labour used for the line and random planting methods, respectively, over the seasons. The lower costs of planting by the seedling throwing method maximised the B:C ratio to 2.85 and 3.14 in the wet and dry seasons, respectively (Table 1). Kandasamy et al. (1996) also stated that as a result of yield maintenance and savings in planting labour costs, the B:C ratio was higher with the seedling throwing method, especially in the dry season. At the Rice Research Station, Kayamkulam, Varughese et al. (1993) also found that the broadcasting of

seedlings reduced the costs of transplanting and increased the net profit over transplanting.

The crop established by throwing older seedlings gave a higher B:C ratio of 3.00 during the wet season and 3.20 in the dry season, due to the higher grain yield. The higher grain yield obtained with higher N application during the dry seaon increased the B:C ratio to a maximum of 3.29 at 180 kg N/ha. Although the B:C ratio was the same (2.85) for both N levels in the wet season, the net income (495 $) was greater at the higher N application rate. An increased number of N splits (4 in the wet season and 5 in the dry season) substantially increased the net income (511 $ and 598 $ in the wet and dry seasons, respectively) and the B:C ratio (2.93 and 3.20 during the wet and dry seasons, respectively) compared to three and four splits in the respective seasons (Table 3).

The results of the two-season study indicate that the throwing of rice seedlings under lowland conditions is not only simple, but also practically feasible and less labour intensive. This practice requires careful water management for the initial one-week period of the establishment stage. The component technologies studied for the seedling throwing method suggest that the use of 30- to 40-day-old seedlings supplied with the recommended dose of 150 kg N ha^{-1} applied either in four or five splits was an appropriate production technology for sustaining the yield and profitability of rice crops established by the throwing of rice seedlings rather than by adopting cost- and labour-intensive transplanting methods.

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EFFECTS OF POWDERED RICE CHAFF APPLICATION ON LODGING RESISTANCE, Si AND N CONTENTS AND YIELD COMPONENTS OF RICE (*ORYZA SATIVA* L.) UNDER SHADED CONDITIONS

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A plot experiment was conducted during 1996 at the experimental farm of the Faculty of Agriculture, Gifu University to ascertain the effects of the application of rice chaff in powdered form (Cp) on the lodging resistance (breaking strength), Si and N contents, grain yield and yield components of rice (*Oryza sativa* L. cv. Hatsushimo) treated with low (L) and high (H) levels of chemical fertilizer under shaded conditions (50%). The results showed that lodging resistance increased in response to Cp application in combination with a L level of chemical fertilizer due to the higher bending moment of the basal internode and the lower lodging index value. Under shaded conditions, the application of Cp with a L level of chemical fertilizer influenced both the yield and yield components of the rice plant, especially the number of panicles m^{-2}, 1000 grain weight and grain:straw ratio, and also tended to increase grain yield. By contrast, Cp application with a H level of chemical fertilizer treatment decreased the yield and corresponding yield components. Under shaded conditions reduced values of yield and yield components were found in rice. Better Si contents could be achieved in rice plants due to Cp application in combination with a L level fertilizer treatment. It thus appears that Cp application has a beneficial effect on rice growth when combined with a L level of chemical fertilizer, and Cp could become an alternative source of inorganic Si fertilizer, forming a sustainable Si recycling system in rice cultivation.

Key words: powdered chaff, lodging resistance, Si, N, yield, shading

Introduction

Rice is a typical silica plant which absorbs a greater quantity of silicon than other cereals (Islam and Saha, 1969; Aleshin, 1988; Kim et al., 1985). Several authors (Okuda and Takahashi, 1964; Mark, 1992; Takahashi et al., 1990) reported that rice requires large amounts of Si for vigorous growth, lodging and disease resistance and to increase yield and yield components, such as the number of panicles, 1000 grain weight, etc. Nowadays, inorganic silicate fertilizers and organic Si materials, such as rice chaff, straw and silicate slags, are frequently applied in many countries including India, South Korea, Taiwan, Hawaii, Cuba and China.

It is well known that the Si content in rice chaff (husk) is higher than in any other part of the rice plant and often reaches about 20% of the mean dry matter content. Unfortunately, the application of rice chaff has not yet become

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standard practice in Japan because of the difficult decomposition of rice chaff in the soil. However, taking into consideration the polluting effect of the excessive use of inorganic fertilizers and the high cost of inorganic Si fertilizers, the use of rice chaff with a high content of Si still seems to be important for maintaining sustainable rice cultivation in rice-growing countries. Similar conclusions were drawn by Sistani et al. (1997). In this study, rice chaff was used in powdered form which is easily decomposed in the soil. Hence our choice of this material, which is currently being wasted (by burning, etc.), as a silicon source. So far, a large volume of research (Ma and Takahashi, 1991, 1993; Takahashi et al. 1990; etc.) has been carried out on silicon release from different silicate materials, such as straw, slags, silicon inorganic fertilizer, etc., and on its uptake by the rice plant. However, the effect of using Cp as a silicon source in relation to rice plant growth, lodging resistance, yield and shading, has received little attention from researchers. The use of shading in our experiment is significant because light is known to play an important role in the growth and yield of rice. Takahashi et al. (1990) reported that environmental conditions such as light intensity, daylength and temperature are also key points in the development of silicon deficiency. Therefore, the present work is an attempt to study the effects of Cp on the lodging resistance, Si and N contents and yield components of rice under shaded conditions.

Materials and methods

1. Experimental setup

The experiment was conducted in the plots of Gifu University, Japan in 1996. The size of each plot was 2.0 × 2.4 m^2 and each plot had two subplots (1.0 × 2.4 m^2). The soil of the plots was a sandy loam. The physical properties (particle size) of the sandy loam soil were sand 82.3%, silt 13.1%, clay 4.6% and water holding capacity 6.68, and the chemical properties (nutrient content) were nitrogen 0.019%, P_2O_5 20.7 mg $100g^{-1}$, K 0.10 mg g^{-1}, CEC 8.20 ml $100g^{-1}$, respectively (Horiuchi et al., 1989). The rice cultivar used was Hatsushimo. The amounts of chemical fertilizers in the H treatment were: 150 kg N ha^{-1}, 200 kg P_2O_5 ha^{-1}, 175 kg K_2O ha^{-1}. The corresponding values for the L treatment were: 60 kg N ha^{-1}, 80 kg P_2O_5 ha^{-1}, 70 kg K_2O ha^{-1}. The total application included basal doses of 60 kg ha^{-1} and 24 kg ha^{-1} of compound fertilizer (N : P_2O_5 : K_2O= 12 : 16 : 14) that were applied 3 days after transplanting to the H and L treatment, respectively. The first top dressing with a rate of 30 kg ha^{-1} at the H level and 12 kg ha^{-1} at the L level each element (N : P_2O_5 : K_2O) was done 3 weeks after transplanting. The N level for both the second top-dressing (8 weeks after transplanting) and the third top dressing (12 weeks after transplanting) was 30 kg ha^{-1} for the H and 12 kg ha^{-1} for the L level, respectively.

A grinder and a sieve with a mesh size of 0.5 mm were used for making Cp from the cultivar Koshihikari. This was applied at a rate of 3 t ha^{-1} two weeks before transplanting. The rice was transplanted with a planting space of 30 × 15 cm on June 20, 1996 and harvesting was carried out on October 20, 1996. A plot without Cp application was prepared as a control. There were 4 replications for each treatment. Details of the treatments are shown in Table 1. Irrigation was carried out with tap water as necessary. The plants were shaded with black cheese cloth to reduce light intensity by 50% from the most active tillering stage to the most active reduction division stage.

Table 1
Details of treatments

Treatments	Abbreviations
T_1. Control* + Chemical Fertilizer–High level	Cont.+C. F. (H)
T_2. Control* + Chemical Fertilizer –Low level	Cont.+C. F. (L)
T_3. Powdered rice chaff + Chemical Fertilizer–High level	Cp + C. F. (H)
T_4. Powdered rice chaff + Chemical Fertilizer–Low level	Cp + C. F. (L)

* No powdered rice chaff was applied

2. Analysis of samples

Lodging resistance was measured mainly on the lower internodes, since these are sensitive to lodging (Hosikawa and Wang, 1990; Wang and Hosikawa, 1991). The 3rd and 4th internodes from the top of the main culms were used for the determination of lodging (breaking) resistance 35–40 days after heading. The lodging parameters were determined as the bending moments (whole plant and basal internode), the lodging index, the section modulus, the bending hardness and the second section moment. These parameters were measured on 5 randomly selected main culms in each subplot. The method adopted for measuring lodging resistance was based mainly on a Tensile tester apparatus (Ookawa et al., 1993).

The top parts of the plant samples were oven-dried and then powdered for Si analysis. The Si contents were determined by the colorimetric molybdenum blue method (Okuda and Takahashi, 1961). Harvested plants were also used for total N analysis by the Kjeldahl method and for yield analysis.

3. Statistical analysis

All data were statistically analysed following standard procedures for analysis of variance (ANOVA) and the differences between means were tested using Duncan's multiple range test (Gomez and Gomez, 1984).

Results and discussion

1. Breaking strength (lodging resistance) of the culm

The breaking strength of the 3rd and 4th internodes 35–40 days after heading are shown in Table 2. Among the H levels, the bending moments of the whole plants were higher for the Cont.+C. F. (H) treatment and lower for the Cp+C. F. (H) treatment at both the 3rd and 4th internodes. Among the L levels, the corresponding values were higher for the Cont.+C. F. (L) and lower for the Cp+C. F. (L). The values for the bending moment of the whole plants were not significantly different between the treatments, but for the bending moment of the basal internode, the Cp+C. F. (L) treatment gave the highest values, which were significantly different from treatments given H levels, while the lowest values were obtained for Cp+C. F. (H). The lodging index for the Cp+C. F. (L) was the lowest in all treatments and significantly lower than that of Cont.+C. F. (H). For the other physical properties, such as the section modulus, the bending hardness and the second section moment, there were no significant differences between the treatments.

Table 2
Breaking strength of the 3rd and 4th internodes between 35–40 days after the heading stage as influenced by Cp application under shaded conditions

Internodes	Treatments	Bending moment of whole plant (g.cm)	Bending moment of basal internode (g.cm)	Lodging index	Section modulus (mm^3)	Bending hardness ($kg.cm^2$)	Second section moment (mm^4)
3rd	Cont.+C.F.(H)	505.5 a	840.0 b	0.61 a	3.85	0.10 b	7.4
	Cont.+C.F.(L)	362.0 ab	918.7 ab	0.34 b	5.57	0.14 a	11.1
	Cp+C.F.(H)	420.5 ab	689.0 b	0.62 a	6.55	0.09 b	14.5
	Cp+C.F.(L)	303.8 b	1110.0 a	0.33 b	4.31	0.12 ab	8.2
4th	Cont.+C.F.(H)	665.9 a	1188.7	0.58 a	9.31	0.14	22.3
	Cont.+C.F.(L)	478.2 ab	1146.6	0.42 ab	6.32	0.14	13.2
	Cp+C.F.(H)	550.0 ab	913.0	0.60 a	8.29	0.11	19.9
	Cp+C.F.(L)	411.7 b	1201.0	0.36 b	6.34	0.15	13.1

Means followed by the same letter are not significantly different at the 5% level according to Duncan's multiple range test

The breaking strengths of the internodes were affected by Cp application at the L level, as proved by the lower value of the lodging index (Tawara et al., 1965). A higher Si content was obtained in the Cp treatment, with a L level of fertilizer; consequently, lodging resistance was high in this treatment. H level treatments showed a declining tendency for breaking strength, which is not uncommon, because the efficiency of Si to resist lodging decreases with increasing doses of nitrogen (Idris et al., 1975). The bending moment of the whole plant in the Cp+C.F.(L) treatment showed comparatively lower values due to the short plant height or short length of culms from the broken point to the top of the plant. In contrast, the bending moments of the basal internodes had higher values for the Cp+C.F.(L) treatments. These values also indicated an increase in lodging resistance. Yuan et al. (1988) reported that the bending moment of the basal internode was the best characteristic for testing lodging resistance. Furthermore, Ookawa et al. (1993) reported that changes in the degree of lodging and the lodging index were largely dependent on variations in the breaking strength of the basal internode. Nevertheless, the breaking strength of the 3rd and 4th internodes exhibited lower values under shaded conditions. This phenomenon was clarified by Ookawa et al. (1993), who found that the breaking strength was much smaller in the shade.

2. Yield and yield components

Table 3 shows the results obtained for yield and yield components. The number of panicles m^{-2} was the highest in the Cp+C.F.(L) and lowest in the Cont.+C.F.(L) treatment. Heavy applications of chemical fertilizer did not improve the number of panicles m^{-2}; on the contrary, lower values were obtained both in the control and with Cp. A similar trend was observed for the

number of panicles hill^{-1}. The dry weight of the whole plant showed significantly higher values in H treatments than in L treatments. However, the values for the Cp+C.F.(L) treatment indicated no significant difference from the other treatments applied. Heavy applications of chemical fertilizers, together with Cp or without it, showed decreasing trends in the percentages of ripened grains in the case of shading. Thousand grain weight was the highest in the Cp+C.F.(L) treatment, while Cont.+C.F.(L) also gave higher values than the H treatments. Similar trends were observed for the grain-straw ratio. Cp+C.F.(L) showed a significantly higher value, with the lowest value for Cp+C.F.(H). For grain yield (g m^{-2}) the application of Cp+C.F.(L) showed the best effect, exceeding the value of the Cp+C.F.(H), which decreased the yield, compared with Cont.+C.F.(H).

Table 3
Yield and yield components of rice plants as influenced by Cp application under shaded conditions

Treatments	No. of panicles (m^{-2})	No. of panicles (hill^{-1})	Dry weight of whole plant (g m^{-2})	Percentage of ripened grains (%)	1000 grain weight (g)	Grain: straw ratio	Grain yield
Cont.+C.F.(H)	177.6	8.0	876.3 a	53.6	28.4	52.2 ab	254.7
Cont.+C.F.(L)	154.7	7.0	582.9 b	73.4	29.3	79.3 a	216.8
Cp+C.F.(H)	204.2	9.2	806.0 a	48.9	29.0	51.6 b	229.1
Cp+C.F.(L)	209.4	9.4	786.6 ab	74.1	29.8	81.9 a	295.1

Means followed by the same letter are not significantly different at the 5% level according to Duncan's multiple range test

Under shaded conditions the best values of yield and yield components were obtained with the Cp+C.F.(L) treatment. However, the values of yield and yield components were always reduced by shading. This phenomenon agreed with the reports of numerous authors (Lee et al., 1990; Kato, 1986; Mawaki et al., 1990; Dey et al., 1989; Islam et al., 1992), who indicated that shade (50%) reduced the number of panicles, percentage of ripened grains, 1000 grain weight and grain yield. Moreover, the low yield obtained in this experiment may also have been due to the poor fertility of the sandy loam soil.

The yield components, especially the number of panicles (m^{-2} and hill^{-1}), 1000 grain weight, grain-straw ratio and grain yield, were influenced by Cp application. This influence was clarified by the reports of Jakhro (1986), Ota (1988), Zang (1986) and Lee et al. (1990). However, the experimental values indicates that the yield and yield components decreased in the Cp+C.F.(H) treatment. In the present study the percentage of ripened grains was lowest in the Cp+C.F.(H) treatment. It could be that this reduction in the percentage of ripened grains was brought about by applying high N to the soil. This concept was supported by Matsushima (1980), who found that too great a desire to increase the number of panicles alone can cause the rice plant to produce many non bearing tillers and many small, weak panicles, with a resultant reduction in

percentages of ripened grains and yield. The application of Cp+C.F.(H) did not show any improvement in grain yield; in fact, the grain yield in this treatment was lower than in the control. This was probably due to the release of toxic substances during the decomposition of Cp at the H fertilizer level. In addition, Jakhro (1984) reported that rice chaff in combination with high N did not increase the grain yield, though he used burned and unburned rice chaff. It may be interesting to note that on the contrary, Cp application with L level N gave the best yield and yield components among the treatments. This result was consistent with the report of Park et al. (1989) on the effect of N and Si on the rice yield. However, his results were based on inorganic Si fertilizer. There are no reports about the effect of silicate organic material such as rice chaff with a L level of N on the yield. As a result of this, further work is necessary to show if differences in the experimental results occur when a L level is used.

3. Silicon and nitrogen contents in the plants

Table 4 shows how the silicon contents in the plants under shaded conditions 35–40 days after heading were influenced by Cp application. The results showed that the percentage of Si was highest in the Cp+C.F.(L) treatment and that this value differed significantly from the Cont.+C.F.(H) and Cp+C.F.(H) treatments. In the case of Cp+C.F.(H), the Si content declined significantly compared to Cp+C.F.(L). In contrast with the H level treatments, the Si content in the plants tended to increase at the L level.

The results obtained for Si content showed that the Si content in the plants was extremely low in the Cp+C.F.(H) treatment under shaded conditions, so it seems that at the H level of fertilizer treatment Cp did not improve the uptake of Si by rice plants. Ishizuka et al. (1951) indicated that as more N is added to the culture solution, so the Si uptake by the rice plant decreases. Judging from the results of experiments carried out by various investigators in Japan, it can be concluded that Si application to rice makes it possible to increase the application of N to some extent (Okuda and Takahashi, 1964). One other possible reason for the lower Si content in the rice plant may be the release of toxic substances during the decomposition of Cp. However, this concept needs the support of both morphological and physiological studies in the future. The Cp+C.F.(L) treatment gave the highest Si content under shaded conditions. This is further supported by Idris et al. (1975), who demonstrated that added Si significantly increased the Si uptake by rice plants at lower doses of N.

The percentage of total N in the straw was the same in the control and Cp treatments when a H level of fertilizer was applied (Table 4). In contrast, at L levels the total N content in the straw tended to increase in the Cp treatment and was higher than the control. However, no significant differences were found between the treatments. On the other hand, the total nitrogen content in the grain increased in treatment Cp+C.F.(H) compared with Cont.+C.F.(H), though the values were not significantly different from each other. Similar trends were also observed in treatments with a low level of chemical fertilizers, where Cp+C.F.(L) was higher than Cont.+ C.F.(L). Again, there was no significant difference between the two fertilizer levels.

Table 4
Silicon and nitrogen contents in rice as influenced by Cp application under shaded conditions 35–40 days after the heading stage

Treatments	Silicon (%)	Nitrogen (%)	
		Straw	Grain
Cont.+C. F. (H)	3.2 b	0.56	1.03 ab
Cont.+C. F. (L)	3.6 ab	0.37	0.75 ab
Cp+C. F. (H)	3.2 b	0.56	1.21 a
Cp+C. F. (L)	4.0 a	0.47	0.84 b

Means followed by the same letter are not significantly different at the 5% level according to Duncan's multiple range test

The values obtained for the total N content in the straw and grain of the plants under shaded conditions showed declining tendencies. Tsuno et al. (1989) and Dey et al. (1989) reported that low light (50% sunlight from 40 days after planting to flowering reduced the total N uptake by the plants.

Based on these results, it can be concluded that lodging resistance was high in treatments combining Cp application with a L level of chemical fertilizer under shaded conditions due to the higher bending moments of the basal internodes and due to the lower lodging index values. Cp application at a L fertilizer level is important for attaining a good grain yield and Si content in the rice plant. However, a low yield response to Cp application was found at a H level of fertilizer, so it is evident that a properly balanced use of organic Si material (Cp) and chemical fertilizer is necessary. Finally, the choice of Cp as a silicon organic material is significant for environmental protection and for the quantitative balance of chemical fertilizer.

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ANALYSIS OF IRRIGATED AND NON-IRRIGATED SUGAR-BEET PRODUCTION BASED ON HUNGARIAN DATA

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The goals of our experiment were to compare irrigated and non-irrigated sugar-beet production in respect of varieties, number of plants and harvest time, as well as to determine the extent to which irrigation influences the yield. Farm and field data for the period 1979–1991 served as the basis for the experiment. The region surrounding the sugar factory in Petőháza, where 40% of Hungarian sugar-beet production is concentrated, was analysed in detail. There was no significant difference between the varieties sown in the two production methods. Harvesting time had an effect primarily on the sugar %, and plant density on beet and sugar yield. Changes in plant density and harvesting time caused greater changes in the yield in non-irrigated production than in irrigated production. An 18–20% increase in beet- and sugar yield averages was observed as the result of irrigation. In the case of sugar content there was a decrease of 0.6 sugar %. As a consequence of irrigation the beet and sugar yields became more balanced, and only slight fluctuations occurred. This tendency could also be experienced in the case of sugar %, but to a lesser extent.

Key words: sugar-beet, irrigation, plant density

Introduction

In Hungary irrigation was first employed in sugar-beet production in 1904. Irrigated production has been carried out on larger areas since 1960. In the first half of the 1960s 15% of the sugar-beet production areas were under irrigation on average, while this proportion increased to 30% in the early 1970s (Mihályfalvy, 1976). Between 1980 and 1990 the percentage of irrigated areas decreased to 12%.

The positive effect of irrigation on the sugar-beet yield is shown by both the experimental results and by practical experience. The yield- increasing influence of irrigation is especially significant in droughty years. According to literary data irrigation results in a 20–25% increase in beet yield, and has a similar yield-increasing effect on the sugar yield per hectare (Herke, 1911; Schweickhard, 1923; Bittera, 1929; Eich et al., 1984; Roth et al., 1985; Steinhagen, 1985; Dunham, 1988; Davidoff and Hanks, 1989; Švachula, 1993).

Opinions vary on the effect of irrigation on sugar content (Mihályfalvy, 1964). Some authors reported that irrigation had a disadvantageous effect (Herke, 1911; Klik, 1989; Švachula, 1993), while others observed a beneficial influence (Bittera, 1929; Sajó, 1952; Dunham, 1988).

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One possible explanation for these different results is that the pattern of irrigation (quantity, quality, frequency of irrigation), time of irrigation and the annual precipitation all affect the influence of irrigation. However, there is general agreement on the fact that irrigation carried out inefficiently (too great an irrigation rate, irrigation performed at the end of the growing season) reduces the sugar % significantly.

Materials and methods

An analysis of irrigated and non-irrigated sugar-beet production was carried out based on farm and field level data for the period 1979–1991. The 13 years examined was a relatively "untroubled" period for Hungarian sugar-beet production, which was characterized by mechanised, large-scale production technology of a high standard.

The database on production technology used in the current research represented 88% of the total sugar-beet sowing area, covering 21,000 fields in 742 villages. For more detailed research the relevant data of the Petőháza sugar-beet production region were selected.

In order to investigate the influence of plant density on the yield, the fields were classified according to the plant number at harvest. For each group the average yields (beet production, sugar content, sugar production) and standard deviations were calculated. The relationship between harvest time and yield was evaluated by analysing the average values and standard deviations on a five-day basis. Conclusions were drawn from the results of 1266 fields in the case of plant density, and from those of 2531 fields for harvest times. In both cases the relationships were established by regression analysis.

The yields achieved in irrigated and non-irrigated treatments were compared by applying statistical tests on the mean values for each year (Sváb, 1981).

Results and discussion

The irrigated sugar-beet growing areas in Hungary are shown in Figure 1. according to the regions served by each sugar factory and the number of irrigation years. The main irrigation centres developed in the Petőháza, Szolnok and Kaba regions. About 40% of the total irrigated sugar-beet production was recorded in the Petőháza region and about 20% each in the Szolnok and Kaba regions, while this proportion remained below 5% elsewhere. Nearly 50% of the sugar-beet growing areas were irrigated over the average of 13 years in the Petőháza region.

The highest proportion of sugar-beet production was on meadow alluvial soil, which was the dominant soil type of the region (Fig. 2). The climate of the Petőháza region is moderately dry. The expected amount of rainfall during the vegetation period is less than 350 mm in the eastern part of the region and more than 400 mm in the western part, with 190–200 mm in the drainage season, in July and August (Table 1). In the northern part yields and yield stability significantly depend on the capillary groundwater supply. Thus, the depth and movement of the groundwater (affected by the water output of the Rivers Danube and Rába) play a significant role in crop production. A decrease in the groundwater level causes lower yields; therefore, in dry periods the nearness of the groundwater is particularly important (Palkovits and Schummel, 1992). The gravelled parent rock in the region retains a large quantity of water, making irrigation from wells possible. This phenomenon is generally utilised.

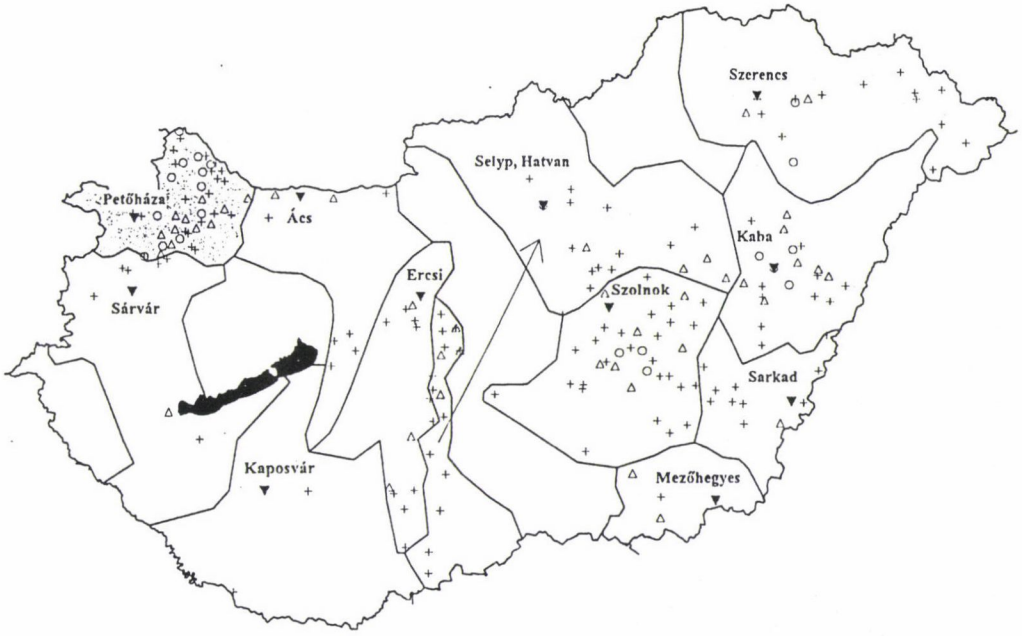

Fig. 1. Hungarian sugar-beet growing areas supplying different sugar factories (1979–1991)
(I. Tirczka and I. Ferencsik)
(Irrigation frequency: + 1–4 years; Δ 5–9 years; o 10–13 years; ▼ Sugar factory; Boundary of sugar factory regions; ■ Study region)

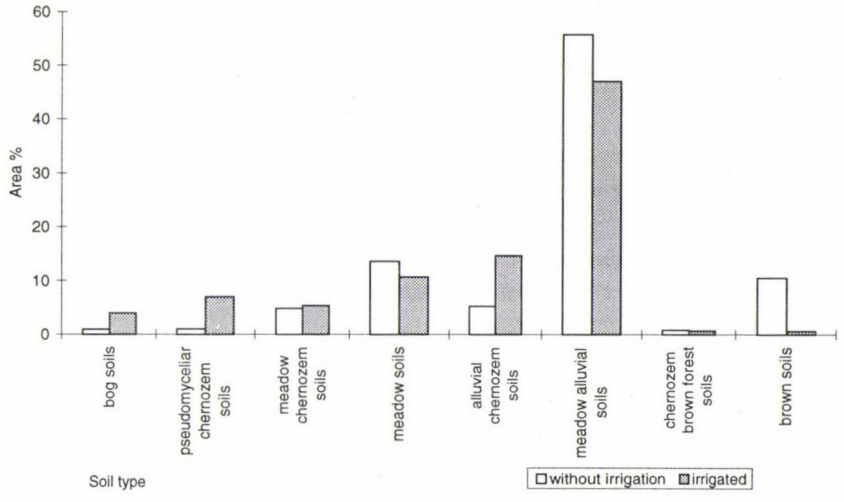

Fig. 2. Distribution of the sugar-beet production area by soil types (1979–1991, Petőháza region)

Table 1
Precipitation conditions in the region of the Petőháza sugar factory (mm)

Years	Months		
	Jan.–Dec.	Apr.–Oct.	Jun.– Aug.
1979	633	376	226
1980	635	457	257
1981	536	384	191
1982	642	433	271
1983	487	302	111
1984	511	336	110
1985	646	366	224
1986	497	327	181
1987	631	395	208
1988	532	315	174
1989	471	394	198
1990	476	335	138
1991	564	382	196
1979–1991	558	369	191
1961–1991	576	383	195
National figures:			
1979–1991	575	374	188
1961–1990	599	392	203

According to literary sources, the selection of the right variety for irrigated production is especially important. The adaptability and responses of the varieties differ in irrigated and non-irrigated environments, and their sensitivity to the quality of irrigation is different as well (Ruzsányi, 1996). An evaluation of farm data on different varieties from 1979–1991 demonstrates that the same varieties were used in both irrigated and dry farm production. This practice should be changed, as the variety range available makes it possible to sow varieties with better water utilisation when irrigation is applied.

Under irrigated conditions 100,000–110,000 plants per hectare is favourable, but in practice significant deviations from this value occurred. In the Petőháza region in the case of irrigation the most frequent plant number at harvest was between 76,000 and 95,000 (on 60% of the total area), while on non-irrigated lands this value was 66,000–90,000 (70% of the growing area). In general lower plant densities (less than 80,000) were found in non-irrigated areas and higher ones (80,000–110,000) under irrigated conditions.

When the plant number at harvest increased, the beet and sugar yields were higher in both types of cultivation (Fig. 3). The yield averages were higher and the standard deviation was lower after irrigation than in the case of dry farming. A change in the plant density had a greater effect on the yield in non-irrigated production. These results are similar to those published by Simon (1984), where an increase in plant density from 70,000 to 90,000 resulted in a 4.8 t/ha yield increase with irrigation, while this increase was 7.1 t/ha in dry farming.

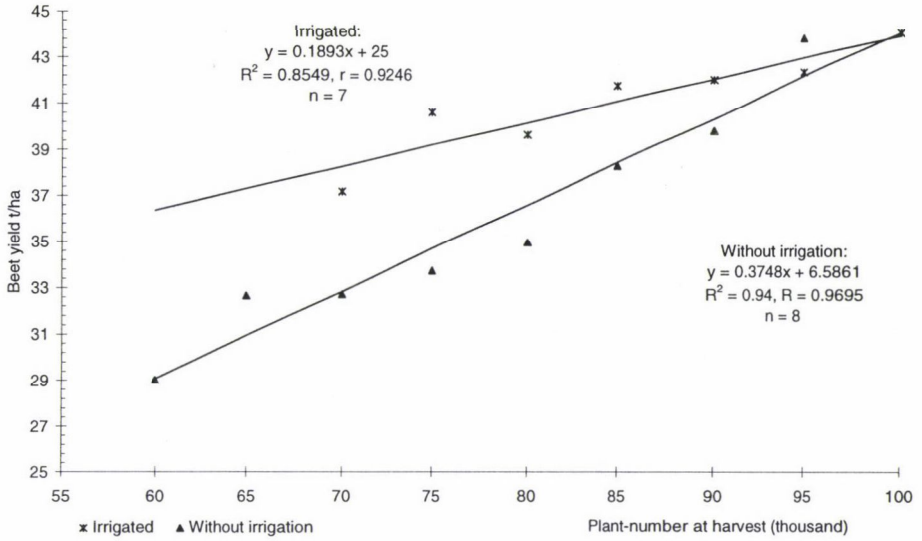

Fig. 3. Effect of plant number at harvest on the beet yield (1979–1991, Petőháza region)

Plant number only affected the sugar % in dry farming: the sugar content increased parallel to the increase in plant number within the 60,000–95,000 range.

The most favourable harvest time for irrigated sugar-beet is 5–20 October. Without a significant quality loss, the beet can be harvested in the 4th–5th week after irrigation (Mihályfalvi, 1976; Ruzsányi, 1996). There was no important divergence in the harvest schedule in the Petőháza region.

Harvest time only had a significant effect on the sugar content (Fig. 4), which increased till the middle or end of October, then decreased. Under non-irrigated conditions the sugar % was higher than under irrigated conditions. A change in the harvest time led to a greater change in the sugar content under non-irrigated than under irrigated conditions. In the case of irrigated production more balanced yield data were obtained.

According to the economic data of the Petőháza region, irrigation increases beet yields (t/ha). In 12 of the 13 years studied irrigation significantly increased the beet yield per hectare (Table 2). The effect of irrigation differed from year to year depending on the agrotechnology applied and on the weather. The difference between the yield data of the two cultivation methods in dry years (1981, 1983, 1984, 1986) was considerable, and fluctuated between 8 and 12 t/ha. Irrigation resulted in average yields 5.5–6.0 t/ha higher (18–20% yield increase) than in dry farming.

Table 2
Beet yields (t/ha) in the Petőháza region (1979–1991)

Year	Irrigated					Non- irrigated					$\bar{x}_1 - \bar{x}_2$	$LSD_{5\%}$
	$\bar{x}_1$	s_1	$s_1\%$	n_1	ha_1	$\bar{x}_2$	s_2	$s_2\%$	n_2	ha_2		
1979	41.54	6.64	16.0	121	5783	35.67	7.74	21.7	120	4079	**5.87**	1.83
1980	41.72	6.45	15.5	78	3992	38.18	7.44	19.5	113	5316	**3.54**	2.05
1981	42.58	6.95	16.3	138	6954	33.43	7.83	23.4	95	3295	**9.15**	1.92
1982	51.46	6.07	11.8	65	3524	46.62	7.90	16.9	146	6579	**4.84**	1.99
1983	39.44	6.01	15.2	86	5002	27.60	7.52	27.2	90	3827	**11.84**	2.04
1984	44.16	8.22	18.6	90	5533	35.81	8.06	22.5	96	4170	**8.35**	2.35
1985	45.25	7.48	16.5	54	3495	40.05	8.16	20.4	134	6401	**5.20**	2.53
1986	42.32	7.99	18.9	65	4367	31.86	7.60	23.9	103	4890	**10.46**	2.43
1987	44.11	5.44	12.3	58	3636	37.76	8.23	21.8	122	5990	**6.35**	2.05
1988	41.89	6.98	16.7	76	4838	35.42	8.82	24.9	105	4948	**6.47**	2.33
1989	39.44	6.72	17.0	51	3402	36.90	8.16	22.1	103	5507	2.54	2.62
1990	34.19	8.44	24.7	107	6130	28.72	8.84	30.8	102	4463	**5.47**	2.35
1991	39.01	6.89	17.8	70	3710	34.72	6.76	19.5	144	6252	**4.29**	1.95
1979–91	41.74	7.97	19.1	1059	60366	36.12	9.24	25.6	1473	65717	**5.62**	0.69

Note: Significant differences in bold

The effect of irrigation on sugar content (%) is more ambiguous. In 10 of the 13 years the sugar content values were lower in irrigated cultivation, but this relationship was only significant in 6 years (Table 3). The sugar content of the beet was 0.6% higher in dry farming. The beet sugar content was only significantly higher (by 0.4%) in two years. The results indicate that irrigation decreases the sugar content.

Table 3.
Beet sugar contents (%) in the Petőháza region (1979–1991)

Year	Irrigated					Non- irrigated					$\bar{x}_1 - \bar{x}_2$	$LSD_{5\%}$
	$\bar{x}_1$	s_1	$s_1\%$	n_1	ha_1	$\bar{x}_2$	s_2	$s_2\%$	n_2	ha_2		
1979	16.90	0.65	3.8	121	5783	17.09	1.03	6.0	120	4079	**–0.19**	0.22
1980	15.54	0.88	5.7	78	3992	15.19	0.84	5.5	113	5316	**0.35**	0.25
1981	15.43	0.92	6.0	138	6954	15.30	1.10	7.2	95	3295	**0.13**	0.27
1982	14.89	0.76	5.1	65	3524	14.45	0.97	6.7	146	6579	**0.44**	0.25
1983	16.98	0.53	3.1	86	5002	17.45	0.82	4.7	90	3827	**–0.47**	0.21
1984	16.18	0.86	5.3	90	5533	16.28	0.74	4.5	96	4170	**–0.07**	0.23
1985	17.75	0.70	3.9	54	3495	18.38	1.03	5.6	134	6401	**–0.63**	0.26
1986	16.96	0.56	3.3	65	4367	17.28	0.69	4.0	103	4890	**–0.32**	0.19
1987	16.97	0.77	4.5	58	3636	17.15	1.00	5.8	122	5990	**–0.18**	0.27
1988	15.15	0.78	5.1	76	4838	15.38	0.76	4.9	105	4948	**–0.23**	0.23
1989	16.12	0.77	4.8	51	3402	16.95	0.93	5.5	103	5507	**–0.83**	0.30
1990	16.36	1.09	6.7	107	6130	16.62	0.95	5.7	102	4463	**–0.26**	0.28
1991	15.36	0.85	5.5	70	3710	16.62	1.10	6.6	144	6252	**–1.26**	0.27
1979–91	16.16	1.13	7.0	1059	60366	16.46	1.45	8.8	1473	65717	**–0.30**	0.10

Note: Significant differences in bold

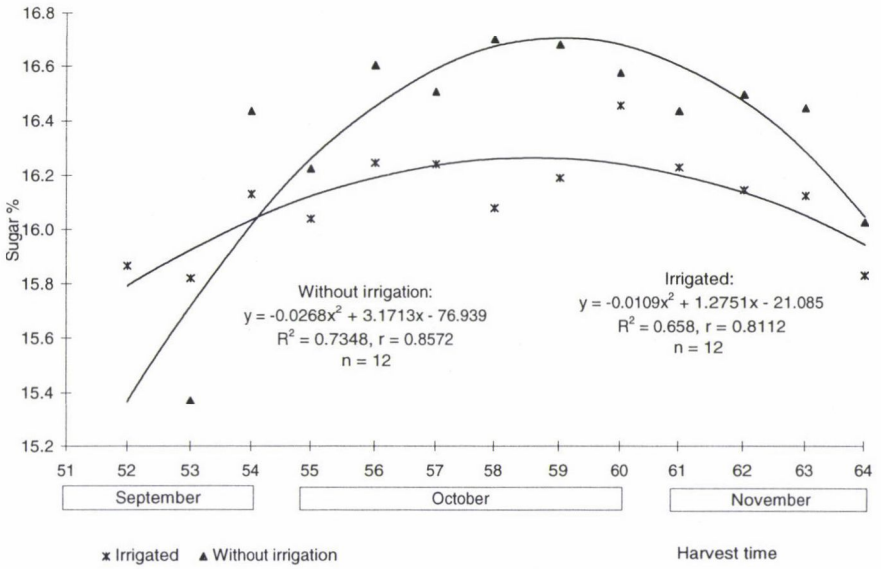

Fig. 4. Effect of harvest date on the sugar content of sugar-beet (1979–1991, Petőháza region)

The effect of irrigation on sugar production (t/ha) is similar to its influence on beet production, since in 11 out of 13 years irrigation increased sugar production by 20% compared with the dry farming results.

According to the variable coefficient values in Tables 2 and 3 the diversity of sugar beet production (quality and quantity) is higher in dry farming than in irrigated cultivation. A comparison of the variable coefficients of the production data leads to the conclusion that the sugar content is less changeable than the beet and sugar yields.

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EVALUATION OF SCROTUM DEVELOPMENT OF CHAROLAIS BULLS OF DIFFERENT AGES IN PERFORMANCE TESTS

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The investigations were carried out in two (**A**, **B**) farms in the years 1992–1994. Charolais bulls (n=375) of different ages were involved in the investigations. The scrotal circumference of the young bulls (sire candidates) was measured at the widest point of the scrotum. The average scrotal circumferences of the Charolais bulls were the following at the various places: **A:** in 1992, age 6–7 months n=50, x=19.3 cm; in 1993, age 13 months n=15, x=33.8 cm; **B:** in 1992, age 7–7.5 months n=51, x=19.8 cm; in 1993, n=21, age 14 months x=35.4, age 18 months x=34.2; n=40, age 9 months x=24.2; in 1994, age 11 months x=30.0, age 13.5 months x=33.4 cm; n=97, age 13.5 months x=33.8 cm. The relationships of the scrotal circumference to body weight and to age were as follows: herd **A** r=0.24–0.65, r=0.11–0.46; herd **B** r=0.22–0.71, r=0.21–0.72, respectively. The correlation coefficients between subsequent scrotal circumferences were also calculated: herd **A**, r=0.57–0.91; herd **B**, r=0.37–0.87. This correlation between subsequent scrotum measurements suggested the possibility of reducing the costs of performance tests.

Key words: young bulls, subsequent scrotum measurements, scrotal circumference, reducing the costs of performance test

Introduction

Reproductive traits in females have been thought to be moderately heritable, but due to the low selection intensity, little improvement through selection can be expected. Because of the higher selection pressure in males, it may be advantageous to select for reproductive efficiency on the male side. Land et al. (1979) have shown changes in female reproduction following selection for testes size in sheep, and this was also found in cattle (Moser et al., 1996).

Scrotal circumference has been found to be positively correlated with several growth and reproductive traits. Hahn et al. (1969), Laszczka and Wierzbowski (1984), Belloir et al. (1984), Zhang et al. (1993) and Gábor et al. (1997) found that scrotal circumference was positively correlated with total sperm production. Brinks et al. (1978), Temblador and Gonzalez (1988) and Polupan (1994) calculated a positive genetic and phenotypic correlation between scrotal circumference and semen quality. Scrotal circumference has been found to be negatively correlated with the age of puberty in the female (Brinks et al., 1978; King et al., 1983; Vargas et al., 1997) and positively

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correlated with pregnancy rates and with the age at first calving in females (Toelle and Robinson, 1985; Smith et al., 1987). Several studies have demonstrated a moderate to close genetic correlation between scrotal circumference and growth traits (Knights et al., 1984; Bourdon and Brinks, 1986; Nelson et al., 1986) and also with weaning weight (Keeton et al., 1996). Bourdon and Brinks (1986) suggested that the positive genetic correlation between scrotal circumference and growth traits would also affect inherent fertility in the females.

Most of the previous reports indicate that the heritability of scrotal circumference is moderate to high in magnitude (Coulter and Keller, 1979; Neely et al., 1982; Latimer et al., 1982; Lunstra et al., 1988; Kriese et al., 1991; Gregory et al., 1995; Keeton et al., 1996; Shepard et al., 1996).

Very few authors in Hungary have so far investigated the development of the scrotum and testicles (Balika et al., 1976; Asem, 1980; Varga, 1990; Tőzsér, 1991, Gábor et al., 1995; Tőzsér et al., 1996).

The objectives of the research were:

- To measure the average scrotal circumference of Charolais bulls at different ages in self-performance tests.
- To describe the relationships existing between scrotal circumference and age, on the one hand, and scrotal circumference and body weight, on the other hand.
- To calculate the correlation coefficients between subsequent scrotal circumferences.

Materials and methods

The investigations were carried out on two (**A**, **B**) farms in the years 1992–1994. Charolais bulls (n=375) of different ages were involved in the investigations. The scrotal circumference of the young bulls (sire candidates) was measured at the widest point of the scrotum (Taylor, 1984).

To describe the relationships between scrotal circumference, age and body weight, the method of correlation analysis was used.

Results

A summary of the average age, body weight and scrotal circumference of the Charolais bulls is shown in Table 1. The average scrotal circumferences of the bulls were the following at the various places: **A:** in 1992, age 6–7 months n=50, x=19.3 cm; in 1993, age 13 months n=15, x=33.8 cm; **B:** in 1992, age 7–7.5 months n=51, x=19.8 cm; in 1993, n=21, age 14 months x=35.4, age 18 months x=34.2; n=40, age 9 months x=24.2, in 1994, age 11 months x=30.0, age 13.5 months x=33.4 cm; n=97, age 13.5 months x=33.8 cm.

Table 1
Body weight, age and scrotal circumference of young Charolais bulls in the years 1992–1994 (mean ±SD)

Herd	Year	No. of individuals	Body weight (kg)	Age (days)	Scrotal circumference (cm)
A	1992	50	240.0±37.2	203±17.0	19.3±1.8
	1993	15	531.3±30.6	410±09.5	33.8±2.6
B	1992	51	246.4±44.6	229±55.4	19.8±2.5
	1993	21	534.7±54.7	430±38.5	35.4±2.2
	1993	21	640.2±49.5	550±46.3	34.2±1.7
	1993	40	337.1±54.4	274±37.8	24.2±2.6
	1994	40	439.6±49.3	344±37.8	30.0±2.3
	1994	40	537.4±51.5	407±37.8	33.4±1.8
	1994	97	513.6±59.3	407±42.4	33.8±2.4

The relationships of the scrotal circumference to body weight and to age are shown in Table 2. The correlation values varied between the following: herd **A:** r=0.24–0.65, r=0.11–0.46; herd **B:** r=0.22–0.71, r=0.21–0.72.

Table 2
Relationship of the scrotal circumference to body weight and age

Herd	No. of individuals	Correlation coefficients of the scrotal circumference to	
		Body weight (kg)	Age (days)
A	50	0.65***	0.46**
	15	0.24	0.11
B	51	0.71***	0.63***
	21	0.69**	0.52*
	21	0.30	0.21
	40	0.81***	0.81***
	40	0.66***	0.72***
	40	0.22	0.26
	97	0.65***	0.42***

Levels of significance: *= P<0.05; **=P<0.01; ***=P<0.001

In these Charolais populations the correlation coefficients between subsequent scrotal circumferences were calculated as: herd **A**, r=0.57–0.91; herd **B**, r=0.37–0.87 (Table 3).

Table 3
Correlation coefficients between subsequent measurements of scrotal circumference

Herd	Measurements	I.	II.	III.	IV.
A	II.	0.81***	–	–	–
n=15	III.	0.66***	0.89***	–	–
	IV.	0.67**	0.81***	0.75***	–
	V.	0.57*	0.80***	0.77***	0.91***
B	II.	0.60*	–	–	–
n=21	III.	0.82***	0.63**	–	–
	IV.	0.70***	0.48**	0.84***	–
	V.	0.77***	0.62***	0.83***	0.87***
n=40	II.	0.75***	–	–	–
	III.	0.37*	0.62***	–	–

Levels of significance: *= P<0.05; **=P<0.01; ***=P<0.001

Discussion

The database available in Hungary on the scrotal circumference of young breeding bull candidates is rather small, so any results obtained in this respect could be a useful contribution to the elaboration of a standard for scrotal circumference in the Charolais breed.

The mean values observed for scrotal circumference in Charolais bulls at 6–7 months of age (n=50, x=19.3 cm) and at 7–7.5 months of age (n=51, x=19.8 cm) were similar to the data published by Coulter (1982) (at age 6–7 months, x=20.0 cm). Coulter (1982) proposed preserving the young bulls which had a scrotal circumference > 20.0 cm.

The correlation coefficients in Table 2 suggest that the effect of body weight and age on the scrotal circumference was similar. The phenotypic correlation between scrotal circumference and body weight was in agreement with the results of Schramm et al. (1989) and Pratt et al. (1991), but contrasted with the results of Knights et al. (1984) and Bourdon and Brinks (1986).

Polupan (1994) calculated a very high reproducibility (n=90 bulls, r=0.89) of the scrotum circumference between 12 and 15 months of age. Neely et al. (1982) and Schramm et al. (1989) observed positive correlation coefficients (Hereford: r=0.49; Angus: r=0.53; Charolais: r=0.70; Simmental: r=0.47) between on-test and off-test values for scrotal circumference.

The positive but not equally close correlations detected between subsequent scrotum measurements, e.g. r=0.62–0.80 betweem the 2nd and the last (3rd or 5th) measurement, suggest the possibility of reducing the costs of performance tests through the safe culling of individuals with a small scrotum at the beginning of the tests.

Conclusions

1. To judge reproductive status, the scrotal circumference of breeding bull candidates should be measured out during self-performance tests.
2. Body weight and age had similar effects on the development of the scrotum.
3. The results of correlation analysis between subsequent (second and last) scrotum measurements suggested that the costs of performance tests could be reduced because of the possibility of early selection.

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Short communication

INFLUENCE OF IRRIGATION AND WEED CONTROL METHODS ON YIELD AND QUALITY OF CASSAVA

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Field experiments conducted at Tamil Nadu Agricultural University, Coimbatore for two years (1994–95 and 1995–96) revealed that drip irrigation with 100% of the surface irrigation water led to a higher cassava tuber yield (39.1 and 38.3 t ha^{-1} in the first and second year of study, respectively) due to continuous moisture availability and higher nutrient absorption. Herbigation (38.9, 38.8 t/ha) and hand hoeing and weeding (39.2, 39.0 t/ha) were comparable in giving higher productivity and quality in cassava. Quality parameters such as starch (33.3, 33.0% in the drip system of irrigation-I_2) and the total sugar content of the tubers (2.25, 2.40% in surface irrigation-I_1) were highly influenced by both irrigation and weed control methods in cassava.

Key words: cassava, drip irrigation, pre-emergence, pendimethalin, herbigation

Introduction

The area under cereals is declining and cash crops are gaining importance in the agricultural product market. Among the cash crops, cassava is known to produce more starch, mainly to meet individual needs. The adoption of micro-irrigation systems may help to increase the productivity and water use efficiency of cassava. Drip irrigation has proved its superiority over other methods owing to the direct application of water to the root zone. In cassava, weeds cause 40 to 90% yield and nutrient losses under irrigated conditions. Cassava, which is planted with wide spacing and has slow growth in the initial stage, provides the chance of early and severe competition by weeds. The control of weeds through chemical means is the only alternative, owing to the escalated cost of labour and its scarcity due to urbanisation and industrialisation. Herbicide applied by spraying may drift away, leading to reduced efficiency. To overcome this, the application of herbicides through the irrigation water (herbigation) is a novel technique which could increase herbicidal efficiency and reduce the cost of application (Iruthayaraj, 1991). With this in view an attempt was made to study the efficiency of herbigation in drip and surface irrigation systems in cassava.

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Materials and methods

Field experiments were conducted at Tamil Nadu Agricultural University, Coimbatore during 1994–95 and 1995–96 to find effective and economic irrigation and weed control methods for cassava. The soil of the experimental field was clayey with moderately good drainage. The soil was low, medium and high in available N, P and K, respectively. The cassava variety CO_2, with a duration of nine months, was selected for the study. The details of the experiments are:

Irrigation methods in vertical strips

I_1: Surface irrigation ad 0.45 IW/CPE ratio (Total - 117.7 cm water in 17 irrigations)
I_2: 100% of surface irrigation water (117.7 cm) through drip (40 minutes daily@4 l/h)
I_3: 50% of surface irrigation water (117.7 cm) through drip (20 minutes daily @ 4 l/h)

Weed control methods in horizontal strips

W_1: Pre-emergence application of pendimethalin at 1.0 kg ha^{-1} by spraying followed by hand hoeing and weeding at 30 and 60 days after planting (DAP).
W_2: Pre-emergence application of pendimethalin at 1.0 kg ha^{-1} through irrigation water (herbigation) followed by hand hoeing and weeding at 30 and 60 DAP
W_3: Hand hoeing and weeding at 20, 40 and 60 DAP
W_4: Unweeded check.

The experiments were laid out in a strip plot design with three replications accommodating irrigation methods in vertical strips and weed control methods in horizontal strips. The cassava sets were planted at the top of the ridge with a spacing of 75 × 75 cm in ridges and furrows. Recommended doses of fertilisers were applied to the cassava crop. For scheduling irrigation based on the climatological approach, the evaporation rate from the USWB Class A Open Pan Evaporimeter at the Agricultural Meteorological Observatory was recorded every day. The amount of irrigation water let into each plot was maintained at 6 l/s using a constant discharge irrigation module fixed in the experimental field. The depth of irrigation was five cm. The drip system installation was designed with measured paths and lengths of main, sub-main and lateral lines with water resources and experimental site. The pressure measured in the main, sub-main and lateral lines was 1.5, 0.75 and 0.5 kg cm^{-2}, respectively. Pre-emergence herbicide, pendimethalin, was sprayed three days after planting using a hand-operated knapsack sprayer fitted with a deflector nozzle. The calculated quantity of herbicide was applied through the irrigation water in both the surface and the drip system. The yield of fresh tubers harvested from the net plot area was recorded.

Quality parameters, namely the starch and total sugar contents in the tubers and the H content in tubers and rind, were estimated. The data were statistically analysed following the procedure described by Panse and Sukhatmne (1985) for a strip plot design.

Results and discussion

Effect of irrigation methods

Irrigation methods significantly influenced the tuber yield of cassava (Table 1) during both the years of study. Irrigation given through a drip system with 100% of the surface irrigation water or by surface irrigation at a 0.45 IW/CPE ratio produced a higher tuber yield compared to irrigation through a drip with 50% of the surface irrigation water. A decrease in the synthesis of metabolites, a reduction in the translocation of nutrients from soil to plant and within the plant, and a decrease in cell division and elongation could be considered

Table 1
Effect of treatments on tuber yield (t/ha) of cassava

Treatment	1995				1996			
	I_1	I_2	I_3	Mean	I_1	I_2	I_3	Mean
W_1	34.9	39.2	32.5	35.5	35.9	38.1	33.1	37.5
W_2	37.4	43.1	36.2	38.9	38.3	42.2	36.0	38.8
W_3	37.6	43.4	36.5	39.2	38.5	42.0	36.6	39.0
W_4	28.0	30.8	22.6	27.1	28.3	30.8	26.9	28.7
Mean	34.5	39.1	31.9	–	35.2	38.3	33.2	–
CD (P=0.05)								
I		0.38					0.33	
W		0.50					0.39	
W × I		0.84					0.75	

as the main causes of depression in the grain yield of sorghum plants when less water was supplied at longer irrigation intervals (El-Bagoury et al., 1984). This was reflected in the tuber yield of cassava irrigated with I_3 in both the experiments, as previously observed by Ayyasamy and Chinnusamy (1996) in cassava. The increase in tuber yield using I_2 and I_1 over I_3 was 11.8 and 18.4% in the first year and 8.1 and 13.3% in the second year of experimentation, respectively. The increase in yield components, such as number of tubers, tuber weight, tuber length and girth, increased the tuber yield of cassava under I_2 and I_1 due to adequate moisture availability and the effective utilisation of nutrients throughout the crop growth period. This is in close agreement with the findings of Ayyasamy and Chinnusamy (1996) in cassava and Mohamed Ali et al. (1974) in groundnuts.

Irrigation applied to the surface at a 0.45 IW/CPE ratio (I_1) or through a drip (I_2) increased the starch and total sugar contents in the tubers, whereas the poisonous chemical HCN (hydrogen cyanide) contents in the tubers and rind were reduced due to the frequent and adequate moisture supply in these treatments in both the years of study. The increased starch (33.3, 33.0% in I_2 in years 1 and 2, respectively) and total sugar content (2.25, 2.40% in I_1 in years 1 and 2, respectively) and decreased HCN content of cassava could be attributed to adequate moisture availability and better crop growth (Table 2). Irrigation improved the quality of the tubers, particularly by increasing the starch content and reducing the HCN content, as observed by Ravindran and George (1988). The net seasonal income and the benefit to cost (BIC) ratio was maximum under I_2 (60,980 and 59,380 rupees in years 1 and 2, respectively) as compared to I_1 and I_3 (Table 3).

Table 2
Starch, HCN and total sugar content in cassava tubers

Treatment	Starch content (%)		HCN content (μg g^{-1})		Total sugar content (%)	
	1995	1996	1995	1996	1995	1996
I_1W_1	32.8	30.7	26.75	25.15	2.27	2.36
W_2	33.8	31.9	26.70	25.72	2.27	2.48
W_3	33.9	33.1	26.49	26.27	2.30	2.51
W_4	30.8	30.0	27.35	27.08	2.17	2.26
I_2W_1	33.2	32.3	27.28	25.93	2.22	2.29
W_2	35.0	33.9	27.40	26.38	2.26	2.33
W_3	35.0	35.1	27.24	26.25	2.23	2.41
W_4	30.0	30.7	29.59	27.26	2.13	2.12
I_3W_1	29.9	29.2	26.82	25.23	1.78	2.06
W_2	30.3	30.7	26.92	26.06	1.87	2.01
W_3	30.4	31.8	26.39	26.07	1.93	1.95
W_4	28.7	28.2	27.64	28.90	1.59	062
CD (P=0.05)						
I	0.45	0.29	NS	0.238	0.060	0.032
W	0.63	0.51	NS	0.892	0.029	0.035
W × I	0.68	NS	NS	NS	0.042	0.044

NS –non significant

Table 3
Benefit-cost ratio of cassava crops

Treatments	Net Seasonal Income (Rupees/ha)		B:C ratio	
	1995	1996	1995	1996
I_1	53150	54450	4.35	4.41
I_2	60980	59380	4.54	4.45
I_3	46580	49180	3.70	3.86
W_1	58300	58700	5.59	5.62
W_2	65230	65030	6.19	6.17
W_3	65480	65080	6.06	6.00
W_4	44860	48060	5.80	6.14

Cost of labour: Men: 50 rupees/8-h day; women: 30 rupees/day
Cost of drip system: 4000 rupees/year.

Effect of weed control methods

During the first year of experimentation each weed control method differed significantly from the other methods in the tuber yield recorded. During the second year herbigation (W_2) and spraying (W_1) were on par. Hand hoeing and weeding (W_3) if provided at the early stage of crop growth were able to increase the cassava tuber yield tremendously. The application of herbicide through the irrigation water (W_2) was also found to increase the tuber yield

significantly over herbicide spraying and the unweeded check. The tuber yield of cassava was increased by 30.8 and 30.3% and by 26.9 and 26.0% in W_3 and W_2 over W_4 during the first and second years of study, respectively. This might be due to the effective utilisation of nutrients because of the conducive environment provided by these treatments. Yield losses due to weeds ranged from 40 to 68% (Akobundo, 1980).

Quality parameters, such as the starch content and total sugars of cassava tubers, were highly influenced by the weed control treatments. Effective control of weeds through herbicide spraying (W_1), herbigation (W_2) and hand hoeing and weeding (W_3) improved the quality parameters of cassava. The starch content (33.1, 33.3% in years 1 and 2) and total sugar content (2.15, 2.29% in years 1 and 2, respectively) were high in the hand weeding and hoeing treatment. Similar findings were reported by Jagannathan (1996) in cassava. A higher monetary return (65,480 and 65,080 rupees in years 1 and 2, respectively) was realised in the hand hoeing and weeding treatments (Table 3), whereas the highest B:C ratio was reported in the herbigation treatment (6.19 and 6.14, respectively in years 1 and 2).

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Review

POLICY MAKING AND RESEARCH IN HUNGARIAN AGRICULTURE*

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In the present state of Hungary's agriculture it is useful to reconsider the relation between policy formulation and research activities. In Hungary the contributing scientific sphere is mainly interested in government policy activities at the lower and upper critical borderlines of state intervention. At the lower critical level and below that, the government has to intervene with the intention of rearranging income flows, input resources and outputs for the benefit of the agricultural sector.

Looking back on the 1960s and 1970s it can be stated that in the first two-thirds of the 1960's Hungarian agriculture was below the lower critical level, while in the approach to the last third of the 1970s the upper critical borderline was not reached at all. Nowadays the Hungarian agricultural sector is well below the lower critical borderline. The reason is no longer the overstrained, forced and distorted collectivization, but privatization, aimed at compensation procedures with the same attributes: overstrained, forced and distorted. To reach and get above the lower critical line there is a need for governmental intervention.

Key words: policy theory model, critical border line, government intervention

Introduction

The present occasion as well as the recent crumbling of ownership structures and incentives in our national economy, specifically inside the agribusiness sector, together with the debts accumulated over recent decades by the agricultural economic profession inspire me to reconsider the relation between policy formulation and research principles and activities.

Theory and model building

We can start with the policy theory model of Ian Tinbergen (Tinbergen, 1956; Fekete, 1965) (Fig. 1). Science, – especially scientific research, the educational and other information originating from it, and the intellectual atmosphere, above all the social productivity and efficiency of the research activities, show their influence in each part of the model; for instance:

a) the set of political instruments, as so-called "outside variables", is present in activities organized on a social scale (by academic and educational institutions);

*A tribute to Imre Dimény, Hungarian Minister of Agriculture 1967–1975, presented at a Joint Session of the Agrarian-Technical and Agricultural Economics Committees of the Hungarian Academy of Sciences, 15 October 1997.

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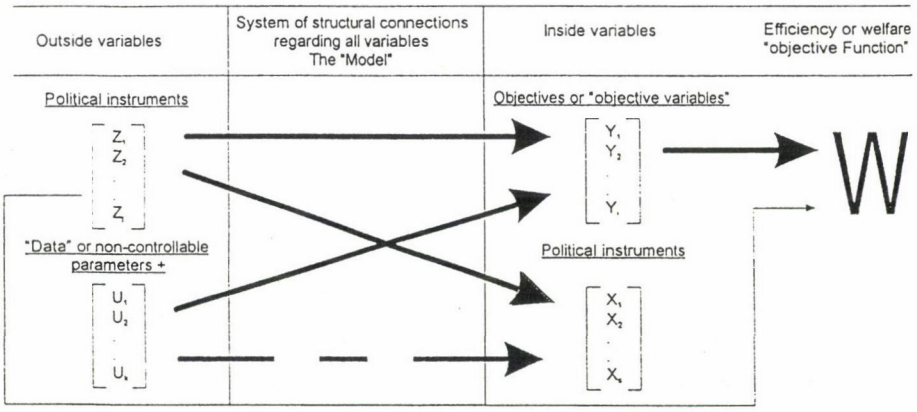

Fig. 1. Unified model of policy making (Not controlled at the level of the political or governmental institutions which set the major goals.) Source: Tinbergen (1956)

b) company-financed private research and spontaneous research and education are placed in the same column among the parameters as non-controllable "variables";

c) the transformation sphere connecting the outside variables (including the restrictions) with the inside variables is found in the second column of the model;

d) science and research also make up part of the "objective function vector" in some way, although positive signs of this can scarcely be perceived in Hungary recently;

e) in this same third column, amongst the "side effects", however, the political effects on science and research may be very much in evidence, just at present – at least partially – in the form of the cause and effect chain of the decline and depression;

f) finally, science is designated as forming part of the general principles and of the highest level of objectives associated with.

This should be updated with one supplementary remark: the model is definitely closed. It does not consider directly the environment, its sometimes decisive effects, or the globalization and international regionalization so significant nowadays.

Remaining in the field of descriptive model-building, according to the comprehensive book edited by Glenn L. Johnson, who is personally well known in Hungary, the constituents and connections of scientific disciplines, research fields, development spheres and interest groups can be demonstrated by means of puzzle-solving (Johnson et al., 1991) (Fig. 2). It is obvious that science and research can be found in every column here, too, though in different forms. They are present most directly in the first and last columns.

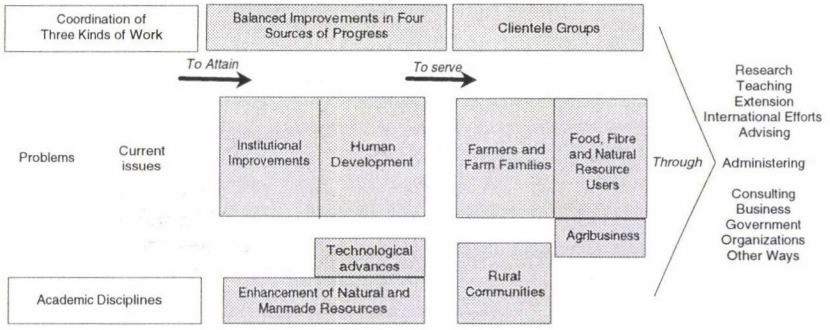

Fig. 2. Building blocks and connecting processes of science and policy-making

Characteristics of the disciplinary priorities of different interest groups

From the viewpoint of utilizable methodology, it is important to determine the demand for research and policies (using a marketing term: the structure of demand) with the help of discoverable priorities.

In the United States the Joint Council on Food and Agricultural Sciences formed a group from the representatives of the overall agrarian profession to carry out a quantitative (ranking) qualification of the major agricultural problem areas (Table 1).

The table shows the priorities declared for the separate agricultural problem areas by each professional group. The points allocated were then added up to provide a ranking. In this way seven professional (disciplinary) groups "ranked" eleven problem areas.

In harmony with other similar studies the following special fields were ranked high indicating the most urgent need and the greatest demand:

1. International agrarian trade and the development policies guided by that;
2. Agricultural marketing in connection with management problems;
3. Financing related to agriculture.

These were closely followed by the structural problems of agribusiness and by price and income issues, though this latter was understandably preceded by the more general problem of agricultural policy. From the accessible publications, it is not clear what kind of content the theory of economics had in the questionnaire and why it was unanimously placed last, reflecting the smallest interest of the so-called "academic" voters.

Table 1
Priorities given to major agricultural problem areas by various agricultural disciplines*

Agents connected with agriculture	Problem Areas										
	FP	AM	AB	P/I	AP	IT	AF	IR	CR.	CE	ET
Farm Suppliers	4	7	6	8	5	9	10	2	1	3	0
Banking/Finance	4	8	6	7	5	10	9	1	3	2	0
Farmers	2	10	7	6	5	8	9	3	4	1	0
Farm retailers	5	9	7	4	6	10	8	1	2	3	0
Consumers	6	5	4	1	3	7	2	8	9	10	0
Academics	4	9	8	5	6	10	7	3	2	1	0
Government	3	9	8	5	7	10	6	4	2	1	0
Total	28	57	46	36	37	64	51	22	23	21	0
Rank**	7	2	4	6	5	1	3	9	8	10	11

*Prioritization on a scale of 0–10, 10 being the highest; **One (1) being the highest; FP – farm production, AM – agricultural market, AB – agribusiness, P/I – price/income, AP – Agricultural policy, IT – international trade, AF – agricultural finance, IR – internal resources, CR – Commercial resources, CR – commercial resources, CE – consumption economy, ET – economic theory. (Source: Johnson et al., 1991)

Typifying of agricultural structures and political aspects of the current agricultural sectors in Hungary

An acceptable typification of agriculture (determining its type) and the characterization of agricultural policy (describing its main characteristics) could promote the assessment of the current situation. It may also serve to ensure the sound targeting of scientific objectives, as well as the correct designation of starting points for rational policies. This can be done in a historical review or using the logics expressed in a publication written exactly three decades ago upon the subject in question (Fekete, 1968) (Table 2).

The most typical features of the agricultural structures currently developing in Hungary can be seen in the long-term as an emerging modernized market economy. The attribute "modern" is synonymous in this context with "coordinated", since it indicates the influencing and controlling functions of the government administration. It is also associated with the term "mixed property" which adds specific content to the substantive features of market economies.

The mixed nature of present Hungarian ownership structures is given by the existence and operation not only of corporate, state and cooperative but also of non-market-oriented, personal and other ownership forms, with a dominance of private ownership (also from a long-term point of view).

Two component subsystems of the present Hungarian agricultural system can be distinguished in principle with regard to their goals, means, structures and wide-ranging effects. One of these is the ever widening sphere of market-oriented companies (including large private farms with access to capital, all

Table 2
Types of farming and characteristic of agricultural policies

Type of farming	Self-supplying (Subsistence)	Commercial (market-oriented)	Company-type	Centrally planned "command" economy	Coordinated (modern) market economy	
					Breadwinner and/or supplementary income earner group	Owner and/or leaseholder, business entrepreneur sector
Main objectives of agricultural policy:	existence-level parity	price parity	income parity	technical parity	rural welfare	profit and annuity, stock-increasing property
Main characteristics of agricultural policy: output (production) maximizing	land area unit	commodity (marketing) volume	company unit	per employee	per capita (family) population	per capital
role of farmer (operator)	breadwinning	selling/trading	profit maximizing	technical manager	breadwinner and supplementary income earner	profit maximizer and/or other modern economic goal follower
mode of optimization	input – output productivity	cost – price	cost – benefit	cost efficiency	reaching the level of aspiration with limited rationality (and information)	maximizing short- and long-term gross income, wealth increase and welfare
lower critical political level	starvation level	fair price level	poverty level	technically obsolescent level	statutory subsistence level	alternative (opportunity) costs
upper critical political level	squandering (wastage) level	maximal price level	luxury level	technical wastage level	aspiration level	average business profit level
main taxation form	head tax, land tax	excise tax, sales tax, customs, duties	income tax	company tax	positive or negative personal income taxes	company and personal income taxes
Change in agricultural population in absolute numbers according to relative magnitude (proportion)	rapid increase	stagnation	decrease	rapid decrease	stagnation	accelerating
	decrease	rapid decrease	decrease	slow decrease	decrease	increase

Source: Classification and forecast by the author according to the methodological scheme presented at the IAAE Conference in 1967 (Sydney)

kinds of cooperative farms, the surviving state farms and the different organizations aimed at integration). The other is formed by the large number of farms providing part-time employment, which are oriented to livelihood, employment and the earning of supplementary incomes. The economic policy aspects of these are also characterized from different directions in Table 2.

At the present time, just like decades ago, the contributing scientific sphere is interested mainly in government policy activities at the lower and upper critical borderlines of state intervention. This intervention takes place mostly in the form of the redistribution of the incomes produced. At the lower critical level and below it the governmental policy must be to intervene with the intention of rearranging income flows, input resources and outputs (commodities and services) for the benefit of the agricultural sectors. Of course, the future return and benefits of these allowances are also counted at each level. At the upper critical level and above (it should be emphasized: there is a political "activity" line, based on social equity, income parity and other similar criteria) the government reallocates and mobilizes resources from agriculture to other branches of the national economy. At present Hungarian economists are working an incalculable distance from this upper critical level.

Looking back on the period examined, however, it can be seen that in the first two-thirds of the 1960s Hungarian agriculture was below the lower critical level. At the time it was thus reasonable to give state subsidies, which led, due to the initiative force of the peasantry, to the positive reaction to cooperative farms, to the reform of the economic mechanism from 1968 onwards, and to the only significant organizational change at national and farm sector level: the establishment of the Ministry of Agriculture and Food, together with the reorganization of the National Board of Cooperative Farms (TOT).

Looking back it is clear that approaching the last third of the '70s, i.e. roughly at the time when the Minister of Agriculture was replaced, the upper critical borderline after which new trends would have been justified had not been reached at all. The major changes carried out at this point were the withdrawal of income from agriculture, the creation of a new balance between state subsidies and taxes, and an increase in price disparity at the expense of agriculture.

Nowadays the Hungarian agricultural sector is well below the lower critical borderline. Now, however, this is not because of overstrained, forced and distorted collectivization, but to privatization, aimed at compensation procedures accompanied by the same attributes: overstrained, forced and distorted in many respects.

If the lower critical level is to be reached and surpassed as soon as possible there is need for governmental intervention, since agriculture itself does not have enough strength and resources to return to the "normal" position. In Hungary agriculture is unable to meet the effective demand at reasonable prices. Agricultural incomes do not provide a decent living standard. At the low level of technical and management efficiency the expenditures in agriculture are

not competitive enough compared with other branches of the national economy or with the agricultural production of other exporting countries.

In this situation policies involving agriculture should emphasize and follow the principle of price parity directly serving a triple set of goals. These objectives are: (a) a large-scale reduction in transmigration and unemployment; (b) to guarantee a normal return for agricultural investments and labour inputs; (c) to counterbalance the dominance of monopoly forces exerting effects on agriculture (in a direct way: by improving the farmers' bargaining position on the market).

The possible roles of scientists in the policy-making process at different levels can be summarized as follows:

a) as experts, exploring the facts and explaining complexities;

b) as advisers, expressing opinions, offering solutions and revealing the consequences of alternative decisions;

c) as managers, submitting, patronizing and protecting the interest they are employed to represent;

d) as arbitrators, considering arguments and counterarguments and endeavouring to find the one best suited to the main objective to be reached with the means available.

Hungarian agricultural economists should be involved in all these duties nowadays.

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Book review

E. P. CUNNINGHAM: History, genetics and the provisioning of mankind. Boyle Medal Lecture, Royal Dublin Society (November 1996)

The Boyle Medal (named after Sir Robert Boyle, a pioneer in experimental science) was founded in 1895 and has been awarded since then by the Royal Dublin Society to recognise research of exceptional merit carried out in Ireland, be it a pure science or the application of science to the arts, agriculture or industry.

In 1997, it was awarded to Edward Patrick Cunningham, who is currently the Head of the Department of Genetics at Trinity College, Dublin. His entire professional career reflects his pursuit of one of the most sacred goals a scientist may have: providing enough food for mankind; enough food in terms of quantity and quality. Professor Cunningham is not a *'laboratory scientist'* working in an ivory tower, but a geneticist who has a clear understanding of the significance and the practical aspects of his work. The years he spent as Director of the FAO Animal Production and Health Division have also contributed to his broad view. In his lecture, given on the occasion of the award, he highlighted his main research topics in the field of quantitative and molecular genetics.

In the first part of his lecture, he directed the spotlight on the greatest challenge faced by mankind: the problem of how to feed almost 12 billion people in the close future, when the world population will be doubled. In a period that spans only the second half of the 20th century, the present explosion seems to be a steady increase. However, in the history of mankind, this (i.e. the period starting with the Industrial Revolution, or even with the dawn of human civilisation) seems more like a wall. Citing the example of predictions in the 1960s, Cunningham states that our planet can feed twice the present population. In the sixties, when the population was half of the present, it was thought that the Earth could not feed a doubled population. But this proved to be wrong, and now, a smaller proportion of the population has to starve. Unfortunately, the security of the food supply, that lies in cereal production, is threatened by the fact that – at least in Asia, where the need is the greatest – no more arable land can be drawn into cultivation. In addition, 80% of mankind will be city-dwellers. Therefore, the only way to avoid a social crisis is to utilise existing farmlands better and to provide a continuous food supply. But this cannot be achieved by returning to extensive agriculture, since production and consumption have already irreversibly separated. He also warned of the damaging policy of developed countries giving food aid to developing countries. The local agriculture collapses as cheap food floods the markets.

Prof. Cunningham then gave an account of his early work in improving the productivity of Irish livestock. He was a pioneer in the development and application of statistical methods and in the linked genetic and economic models.

He introduced the application of a multi-stage index, to avoid the distortion of the data caused by subsequent separate selection.

Addressing the problem of thoroughbred horses, he found that low fertility was not a consequence of inbreeding. After carrying out calculations on the inbreeding coefficients of all the mares in the Stud Book, a recent inbreeding of less than one percent and an accumulated inbreeding of 13% was found. This latter could be offset by culling 8% of the mares for infertility. Cunningham et al. found that the breeding season falls during the least fertile months. If it was started later (i.e. in early summer rather than in early spring the reproduction rate would rise.

Cunningham et al. confirmed that today's thoroughbred population has a narrow genetic base. About 80 studs were included in the Stud Book when it was established in 1791. It now numbers more than half a million world-wide. Three studs, the so-called 'Pillars of the Stud Book', have been recognised as contributing more than 1/4 of the genes in today's thoroughbreds. Cunningham found a fourth one; thus, these four contribute almost one-third of all the genes.

The heritability of track performance was also determined. It was found that about one third of the difference between the track performance of two horses can be attributed to

genetic superiority, and an annual improvement of about one percent was found. However, there was a paradox: there has not been any improvement in the winning times for decades, although genetic changes have taken place. The possible answer is that the best horses have already reached their physiological limit, that cannot be stretched further, Cunningham says.

Although the milk production of indigenous African cattle is much lower than that of European types, their heat tolerance and tropical disease tolerance make them useful partners in crossbreeding programmes. When they were crossbred with Friesians and Jerseys, their milk production doubled, yet they remained stress-tolerant. It was clear that such improvement programmes could take place in the future if the genetic origins of the various breeds were known.

The nature of domestic cattle origins in Africa is unclear, as archaeological data are relatively sparse. The most widely held view is that the humped zebu (*Bos indicus*) and humpless taurine (*Bos taurus*) types of cattle derive from a single domestication 8–10,000 years ago. Cunningham et al. gained new information on early domestication and subsequent cattle migration from the study of variations in mitochondrial DNA (maternally inherited DNA), Y chromosomes (inherited only by males) and microsatellites (displacement loop sequences) in European, Indian, East and West African breeds. These markers proved very useful for investigations of gene flow and admixture in African populations.

Mitochondrial analysis highlighted a marked distinction between European and African cattle on the one hand (*B. taurus* and *B. indicus*) and Indian cattle (Indian *B. indicus*), on the other, providing strong support for a separate origin for domesticated cattle. The genetic difference between these two groups also reflects the length of time since they divorced, which was found to be half a million years. Since we know that domestication occurred about 10,000 years ago, it can be concluded that there had to be a separate domestication in India.

The similarity of European breeds to each other suggests that their divergence happened quite recently. This may mean that the domestication of their common ancestor took place as the technology of farming spread over Europe from the Near East between the 11th and the 6th century BC.

Some of the African cattle, in phenotype, resemble *Bos taurus*, inhabiting the forest and forest margin region, while others are *Bos indicus*, living mainly in drier areas. In areas where a disease called trypanosomiasis is present, *Bos taurus* is more frequent, being resistant to this disease. Cunningham et al. found that while all African cattle (incl. *Bos indicus*) have *Bos taurus* mtDNA, the Y chromosome of the African *Bos indicus* is similar to that of the Indian breeds. However, most breeds showed a mixture of microsatellites of *Bos taurus* and *Bos indicus* origin.

Cunningham thinks that the original *Bos taurus* population was affected by a *Bos indicus* influx from India through Arabia, perhaps with the Arab expansion. It is likely that the gene flow was channelled through the importation of bulls, crossing them to local cows.

In 1990, an emergency drew attention to Africa. The so-called New World Screwworm of Latin American origin appeared in North Africa. In Libya, more than 12,000 animals were affected by this parasite. The effects of a possible spread were unimaginable. The wild and domestic livestock populations would have been severely reduced, not to mention the suffering and possible human loss. In the southern part of the United States, the livestock industry had fought the war with this enemy in the fifties and found a solution: through the distribution of males sterilised by irradiation, the population soon collapsed. Mr. Cunningham was appointed by the FAO as director to organise the defence.

It was clear that in Libya a total eradication was needed to prevent its spread to the south. Because time was running out, some 40 million sterilised males were imported every week from Mexico and distributed systematically over the infested are. The screwworm disappeared by the following season. The cost of the action was $75 million, about one-fiftieth of the value saved in North Africa alone.

For an epilogue, there are no better words than his own:

'There is no calling better calculated to induce a proper sense of humility than the study of genetics.

We now have sufficient knowledge to appreciate the tremendous beauty, order and complexity that lie at the heart of all living matter. We also know enough to appreciate how little we know. In this, as much else in science, we are in fact the cardinal generation. A flood of new knowledge is rolling toward us. Those privileged to live in this and the coming century have the towering task of coping with this tidal wave of the new enlightenment.'

B. BARANYAI and J. DOHY

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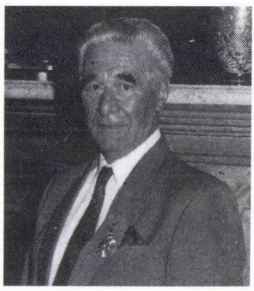
Profile

PROFILE ON STEVEN SALAMON ON THE OCCASION OF HIS 80TH BIRTHDAY



Steven (István) Salamon was born in Hungary in 1918. Soon after obtaining his university degree in Budapest, he was enlisted into the army, then participated in the war and spent three years as a prisoner-of-war in the Soviet Union. After repatriation, he took the position of Research Scientist at the Institute of Animal Breeding, Budapest. Following the oppression of the revolution in 1956, he was obliged to leave Hungary, and spent 12 months in Germany, before migrating to Australia. His successful career there is a good example of how the recipient country has benefited from another's misfortune.

He joined the Department of Animal Husbandry at the University of Sydney in early 1958 in the position of Research Fellow. Soon he was promoted to Senior Research Fellow, then to Senior Lecturer and Reader. Despite his retirement in 1982, he did not give up his research activity. His knowledge of languages and literature made him popular within and outside Australia.

Steven Salamon's scientific interest lies in the field of reproductive biology, mainly of sheep, pigs and goats, the frozen storage of their semen and its subsequent use for artificial insemination. His work on these has gained him an international reputation.

In the early 60s he was a member of the small team which elaborated and introduced into practice the method of oestrus synchronisation in sheep using intravaginal pessaries. The method has facilitated the use of artificial insemination.

His research on the deep freezing of ram, boar and buck semen involved systematic examinations on the composition of diluents, different cryoprotective agents, methods of freezing and thawing and the post-thawing survival of spermatozoa. His intensive laboratory work resulted in the elaboration of extenders suitable for the deep freezing of ram, boar and buck semen.

In subsequent fertility tests it became evident that the low fertility after cervical insemination with frozen-thawed ram semen was due to the poor transport and viability of spermatozoa in the reproductive tract. However, after the surgical insemination of frozen-thawed sperm cells into the uterine horns a

high fertilisation rate was obtained. This led to the use of the laparoscopical insemination of frozen-thawed ram semen in Australia and in other countries.

To examine the effect of long-term storage on fertility, Salamon established a "ram semen bank", from which semen stored frozen for 3, 5, 11, 16 and 27 years has been used for insemination. These tests showed no deterioration in the fertilising capacity of the spermatozoa.

The high fertilisation and farrowing rates obtained after the deposition of frozen-thawed boar semen into the oviducts has refuted the earlier- held belief that boar spermatozoa lose their fertilising capacity during deep freezing. For surgical insemination directly into the oviducts only 0.1–0.2 ml thawed semen is required, making it possible to use one valuable ejaculate for the insemination of several hundred sows.

His work with Angora and Kashmir type goats included not only the freezing of semen, but also the examination of seasonal sperm production in bucks, the hormonal synchronisation of the oestrus cycle and the effect of the method of insemination on fertility in synchronised oestrus.

Salamon's scientific articles and extensive reviews have been published in journals of repute. He is the author, co-author or editor of several books published in English, German and Spanish.

He was very demanding towards himself and those working with him, and has always actively participated in the research projects of his post-graduate students.

In 1993 Steven Salamon was elected external member of the Hungarian Academy of Sciences, and in 1994 awarded the Order of Australia in recognition of his scientific contribution and service to the sheep breeding industry.

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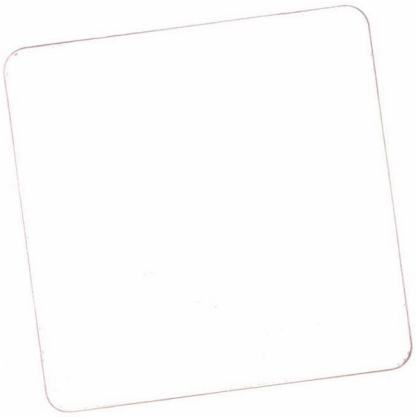

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ORIGINAL PAPERS

SHORT COMMUNICATIONS

Acta Agronomica Hungarica, 46(4), pp.317–325 (1998)

EFFECT OF TEMPERATURE, pH AND HOST PLANT EXTRACTS ON THE GERMINATION OF *CUSCUTA TRIFOLII* AND *C. CAMPESTRIS*

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(Received: 26 June, 1998; accepted: 7 October, 1998)

The pretreatment of *Cuscuta trifolii* and *C. campestris* seeds was found to be effective in increasing the germination ratio of these two dodder species. The removal of the hard seedcoat by sulphuric acid treatment and the conditioning of the seeds promoted the germination of *C. trifolii*. The optimal temperature range for germination in Petri-dishes under controlled conditions is 18–26 °C for *C. trifolii*. In the case of *C. campestris* a high germination ratio (over 60%) was obtained between 16 °C and 32 °C. By studying the effect of different pH on germination it was found that the highest germination ratio for *C. trifolii* seeds could be observed in an acidic pH range, around pH 5.5. When host plant (*Medicago sativa* bv. Verko) extracts were applied to the pretreated, aseptic dodder seeds there was no significant increase in the number of germinated seeds in the case of either *C. trifolii* or *C. campestris*.

By putting the dodder seedlings in close proximity to the host several associations were established and by propagating 6–10 cm pieces from fast-growing parasites it was possible to multiply the parasite.

Key words: plant parasitism; *Cuscuta trifolii* (Bab et Gibs); *Cuscuta campestris* (Yunker); dodder; effect of environment

Introduction

Over 3000 species of flowering plants utilize a parasitic mode of nutrition, yet basic knowledge about their physiology and biochemistry is limited. In contrast to almost all other plants, most of the parasitic angiosperms rely on their hosts for signals that control their initial stages of development (e.g. germination, haustorium initiation) (reviewed by Stewart and Press, 1990). Parasitic angiosperms are generally separated into holo- and hemiparasites. Dodder, an annual holoparasitic flowering plant, is widely distributed in North America and Europe and various species are found on the other continents. It is a very detrimental weed in the alfalfa crop and, where it is uncontrolled, the production of alfalfa seed may be severely reduced and profitability greatly diminished (Graham et al., 1979). The dodder plant reproduces from seed that overwinters in soil or as a contaminant in alfalfa seed lots.

Two *Cuscuta* spp. commonly occur on alfalfa, *C. trifolii* (small-seeded dodder) and *C. campestris* (large-seeded dodder) (Gimesi, 1979). *Cuscuta* species belong to the *Convolvulaceae* family but are yellow instead of green and although some species do have a small amount of chlorophyll they cannot support themselves autotrophically but must live as obligate parasites.

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One of the first key steps in parasitic development is the germination of the seeds. This process may be under the control of a host plant, which secretes a germination stimulant into the soil. The first naturally occurring germination stimulant, strigol, was identified from cotton, the host of *Striga lutea,* a root parasite (Cook et al., 1966). In the case of the *Cuscuta* species no germination stimulant originating from the host plants has been identified (Gimesi, 1987).

Following germination the host must be present for further development. If this is not the case, the *Cuscuta* seedling will die within a short period. Parasites of the *Cuscuta* genus differ with respect to the host range (narrow and broad host range species) (Stewart and Press, 1990). *C. campestris* infection can be observed in various species in the field (carrot, onion, etc.), while the *C. trifolii* host range seems to be limited to alfalfa and clover.

The primary aim of the present experiments was to establish the proper conditions for the germination of *Cuscuta* sp. dodder under laboratory conditions and to obtain information about the effect of environmental factors influencing the germination processes of *Cuscuta* spp. Another goal was to establish a reproductive dodder-alfalfa association under laboratory conditions for further studies on the mechanism of plant-parasite interaction.

Materials and methods

Plants

Seeds of dodders (*Cuscuta trifolii* Bab. et Gibs and *Cuscuta campestris* Yunker) collected at Kompolt, Hungary in 1991 were kindly provided by Prof. I. Bócsa. The alfalfa, *Medicago sativa* bv. Verko, also originated from Kompolt. All seeds were stored at 4–6°C in a dark cold room till use.

Treatment of the seeds

The *Cuscuta* ssp. seeds were pretreated and sterilized according to a slightly modified version of Furuhashi's method (Furuhashi, 1991). The seeds were soaked in concentrated H_2SO_4 for 20 minutes to remove the hard seed coat. Alternatively, the hard seed coat was removed or injured mechanically: the seeds were rubbed against emery-paper in a mortar for at least five minutes. The seeds were subsequently washed with distilled water and sterilized in 1–2% (w/v) sodium hypochlorite for one hour. The NaOCl was eliminated by washing with sterile distilled water at least five times, and after which the seeds were soaked for an additional 1 hour in sterile distilled water at room temperature.

After soaking for 1 hour in water and without further sterilization, the alfalfa seeds were planted into pots (10 seeds/pot) containing soil, and grown in a greenhouse at 23±2 °C under 16/8 hours light/dark conditions.

Test for germination

The pretreated, aseptic *Cuscuta* ssp. seeds were put on solidified culture medium containing Murashige and Skoog's mineral salts (Murashige and Skoog, 1962) supplemented with 800 mg/l NH_4NO_3 as nitrogen source. The seeds on the surface of the medium in the Petri dishes were kept under diffuse light. The germinating seeds were counted every second day for at least three weeks until no more germinated and the percentage of germinated seeds was calculated. Where necessary, additional components at different concentrations were added to the medium in 3 ml agar (0.7% agar) and poured on the top of solid plates prepared in advance.

Extraction of soluble compounds from different alfalfa organs

The roots and the leafy stems of alfalfa grown in soil in the pots were collected separately, measured, cut into small pieces and mixed with one of several solvents. Water, ethanol, a water-ethanol mixture (1:1) and chloroform were applied to extract the soluble materials from the plant organs. An OMNI-MIXER HOMOGENIZER Model No. 17106 was used to homogenize the plant tissues in the solvent. The extraction was carried out with ten one-minute homogenizations, using constant cooling on ice. The mixture was centrifuged for 20 min in a Sorvall RC5 centrifuge using a GSA rotor at 10000 rpm and 20 °C. The supernatant was poured into a fresh tube and centrifugation was repeated. This second supernatant was filter-sterilized and stored at –20 °C in aliquots. The final volume of solvent was calculated on the basis of the amount of plant materials (5 ml solvent per 1 g of fresh plant tissue). The extracts were applied in concentrated and diluted forms.

Results and discussion

Pretreatment of the seeds

Several authors have reported that the pretreatment of matured parasite seeds helps the attainment of the proper conditions for the germination process (reviewed by Stewart and Press, 1990). After ripening in the field the *C. trifolii* seeds require a long period of maturation in the soil (Gimesi, 1987). The nature of the changes occurring in the *Cuscuta* seeds after ripening are unknown. Various structural and metabolic changes in the seed coat and embryo may occur during seed dormancy. Some authors have suggested that changes in respiratory substrates are involved in the after-ripening processes of *Striga* (reviewed by Worsham, 1987).

In the present experiments the seeds of both species of *Cuscuta* were stored at 4–6 °C for the winter. If after this storage the seeds were put directly into pots in the greenhouse to germinate, only a few germinated plants were obtained either in the presence or absence of alfalfa. In the case of *C. trifolii* the germination ratio was 1–2% and the amount of germinating *C. campestris* was less than 10% (Fig. 1). To increase germination, the hard seed coat was removed mechanically or by sulphuric acid treatment before using the dodder seeds. Following this the maximum germination of *C. trifolii* seeds increased to 10–12% (Fig. 1A). The mechanical removal of the seed coat did not increase germination in the Petri dishes (data not shown). In contrast to *C. trifolii,* in the case of *C. campestris* the percentage of germinating seeds was higher without the H_2SO_4 treatment and soaking in sulphuric acid did not increase germination dramatically (Fig. 1B). It was concluded that the conditioning of *Cuscuta trifolii* seeds helps to increase germination, as in the *Striga* system (Stewart and Press, 1990). This treatment clearly influences the processes which enable dormant seeds to become competent for the initiation of germination. It is possible that water uptake through the seedcoat is one of the key steps in this process. Removal or injury of the seed coat provides access by water to the inner tissues of the seeds.

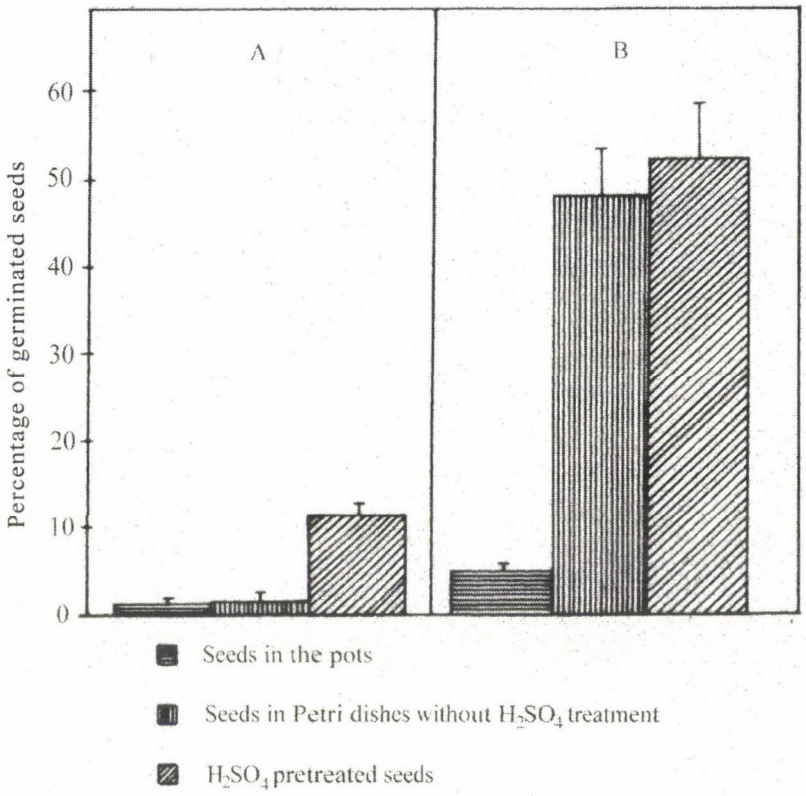

Fig. 1. Effect of pretreatment of *Cuscuta trifolii* (A) and *C. campestris* (B) seeds on their germination in soil at 23±2 °C. In the calculation of percentage of germinated seeds the standard deviation was under 10%

Effect of temperature

The seeds of *C. trifolii* and *C. campestris*, prepared and sterilized according to the Material and Methods, were placed on Petri dishes (100 seeds/Petri dish) and incubated at different temperatures from 8 °C to 32 °C under diffuse light. The percentages of germinated seeds were calculated by counting the seeds every second day for three weeks. After three weeks no changes in the number of germinated plants could be observed. Figure 2A shows that the optimal temperature range for germination was between 20–26 °C for *C. trifolii*. In the case of *C. campestris* a high germination ratio (over 70%) was obtained between 16 °C and 32 °C (Fig. 2B). Consequently, this temperature was set at 23±2°C in subsequent experiments for both species. This temperature is also the best for the germination of *M. sativa*. The effect of temperature on germination may well explain the increase in *C. trifolii* infestation in alfalfa during warm summers in Middle Europe (Gimesi, 1979). Where the climate is cooler (for example in North Europe) *C. trifolii* rarely appears.

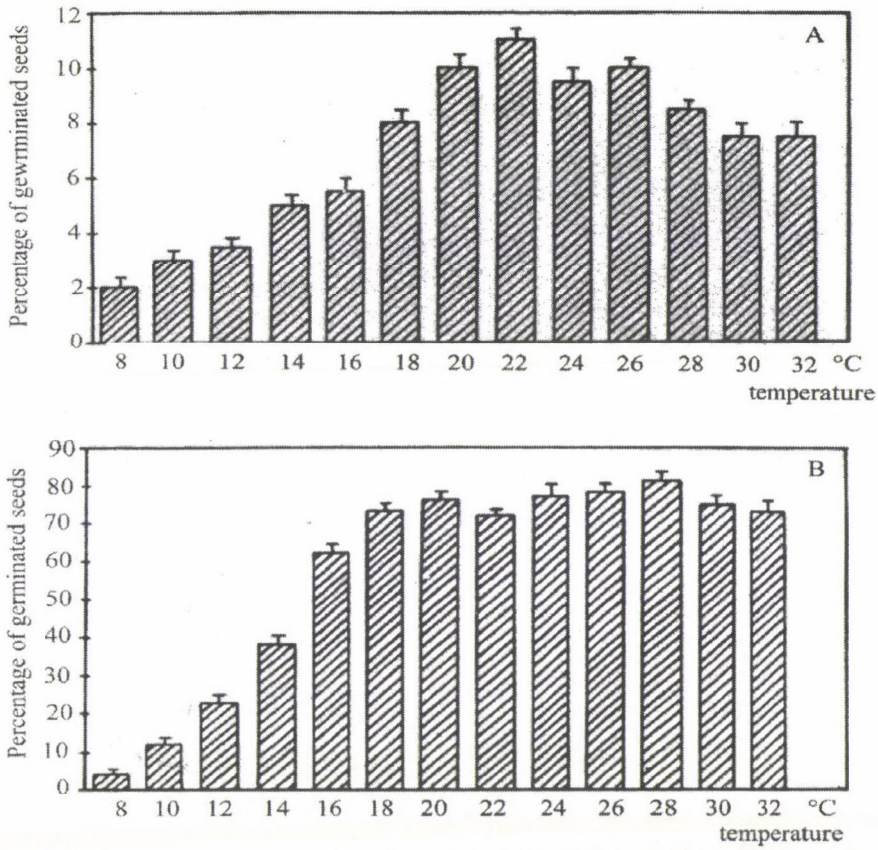

Fig. 2. Effect of the various temperatures on the germination of *Cuscuta trifolii* (A) and *C. campestris* (B) seeds on Petri dishes. Data were calculated from at least five different plant test series in which at least three parallel were applied (the standard deviation was under 12%)

Effect of medium pH on the seeds

The *Cuscuta* ssp. seeds, pretreated as described in the Materials and Methods, were placed on Petri dishes containing MS medium supplemented with nitrogen. The pH of the solid medium in different Petri dishes was adjusted with HCl (0.1 M) or NaOH (2 M) to pH 5.0–pH 9.5 in increments of 0.5 units. The sterile seeds were germinated at 23±2°C for at least three weeks. Figure 3A shows that the highest germination percentage for *C. trifolii* was obtained at acidic pH values between pH 5.0–6.0. Changes in the pH had no effect on the germination of *C. campestris* (Fig. 3B).

These experiments prove that, besides the temperature, the pH is clearly an important environmental factor affecting *C. trifolii* seed germination. The fact that the acidic pH range increased the germination of *C. trifolii* seedlings could be a biological explanation of the heavy infestation of dodder on alfalfa plantations in Central Europe over the last twenty years, since soil acidity has increased as a consequence of environmental pollution, e.g. acid rain.

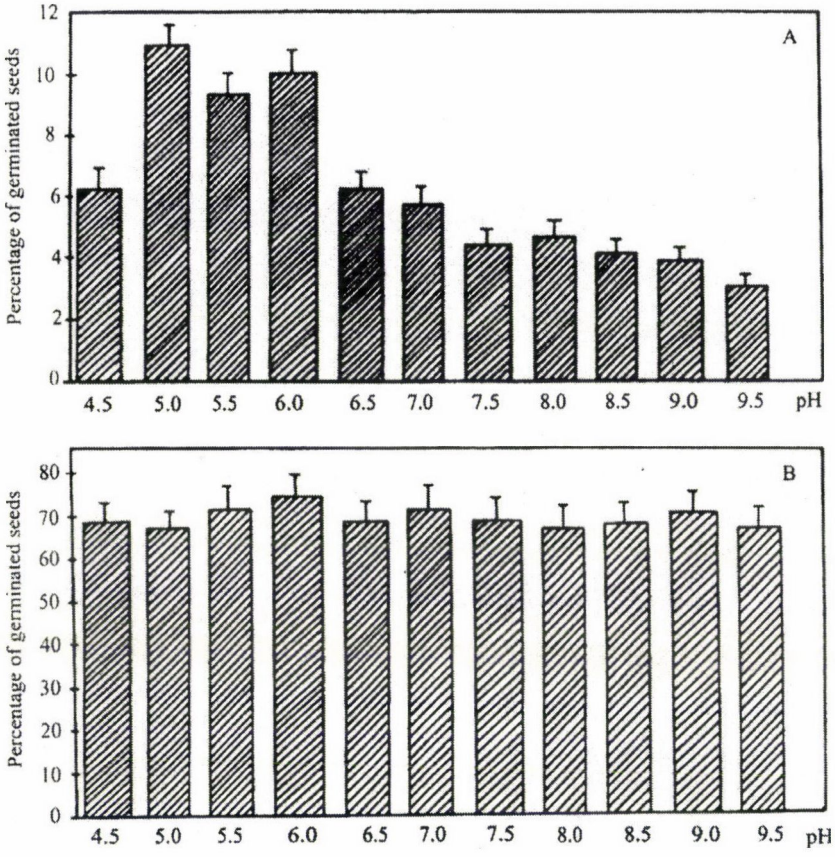

Fig. 3. Effect of the medium pH on the germination of *Cuscuta trifolii* (A) and *C. campestris* (B) seeds on Petri dishes at 23 °C

Effect of host extracts on dodder germination

Different extracts were prepared from the roots and leafy stems of alfalfa according to the Materials and Methods. These extracts were mixed into top agar in 2-, 10-, 100-, 1000-times diluted and undiluted forms and plated out. The pretreated sterile seeds were spread onto the surface of these plates. The seeds were germinated under the above-mentioned conditions for at least three weeks and the number of germinated plants was counted. In the case of *C. trifolii* there was no significant increase in germination after applying host extracts either from the root or from the stem compared to the control plates which did not contain any kind of extracts.

Host extracts prepared with these kinds of solvents did not lead to any significant effects for *C. campestris* either.

These results are in agreement with earlier observations that in the case of *Cuscuta* species no germination stimulants originating from the hosts has so far been identified (Gimesi, 1987).

Cuscuta growth after parasitization on the host in the greenhouse

Three to four days after germination in Petri dishes, small *C. trifolii* seedlings were placed in close proximity to the host plant in the greenhouse at 23±2 °C and under 16/8 hours light/dark period. Using these conditions several dodders parasitized their host. *C. trifolii* surrounded the host stem, becoming stably attached to the epidermis and entering the phloem of the host with its developing haustoria. Having established a parasitic relationship with the host, the *Cuscuta* spread very rapidly and covered the host in the pots within two days. On the basis of experience in the field (Gimesi, 1979) attempts were made to multiply the parasite *in vivo*. Pieces 6–10 cm long were cut from the dodder stem and used to infect a host plant growing in another pot. All the stem pieces successfully attached themselves to the new host and initiated rapid growth. The same infection process was repeated with pieces originating from the "new" generation. With this method "first-generation" and "second-generation" host-parasite interactions could be obtained. The success of this method was illustrated by the flowering and seed set in both "first" (Fig. 4A) and "second" generation plants (Fig. 4B).

Using this simple method unlimited numbers of alfalfa-dodder associations can theoretically be produced, thus avoiding the complicated and expensive *in vitro* culturing of *Cuscuta*. In this way the plants can be kept under permanently controlled conditions in the greenhouse without the danger of accidental release. It is thought that the alfalfa-dodder parasitic relationship will be an excellent system for studying the nature and mechanism of flowering plant parasitism.

Conclusions

On the basis of the experiments it can be concluded that changes in environmental conditions may be one of the reasons why *C. trifolii* and *C. campestris* appear to different extents in the field. They germinate at various ratios according to their germination abilities, which are influenced by the seed coat, pH and temperature conditions. Although there is no direct evidence of the effect of the host plant on the germination process of dodder, it would be too early to exclude the role of the hosts in this process. A reproducible and effective system has been established to study the plant-parasite interaction under laboratory conditions.

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The author would like to thank Dr. C.W.V. Bachem for his critical reading of the manuscript, Ms. E. Maszlag and Ms. Zs. Kotsis for their excellent technical assistance. Appreciation is also expressed to Prof. I. Bócsa for providing the alfalfa, *C. trifolii* and *C. campestris* seeds. This work was partially supported by grants from the National Committee for Technological Development (OMFB Grant No. 92-97-16-0122) and the Hungarian Scientific Research Fund (OTKA Grant No. F-5047/1992).

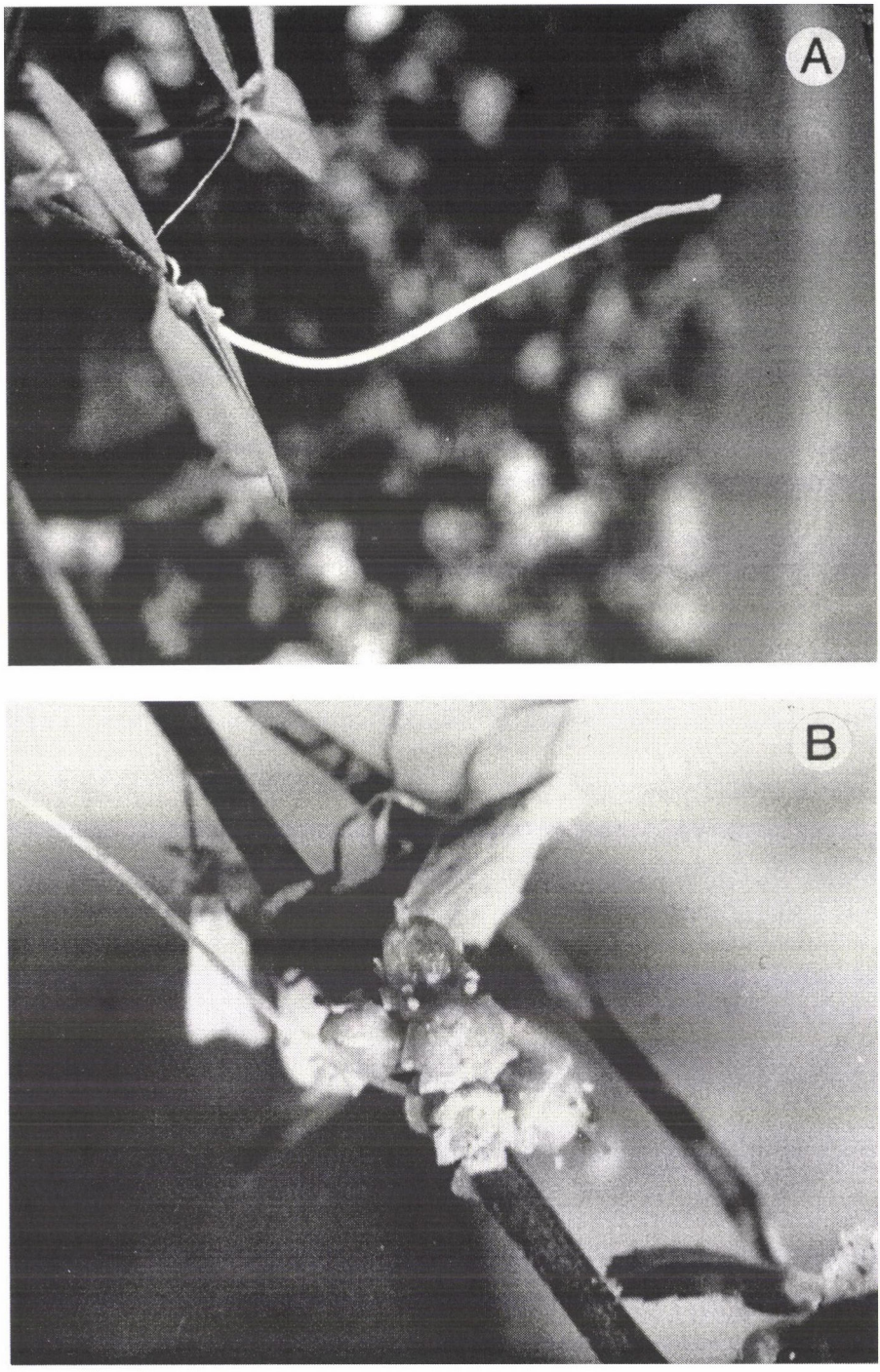

Fig. 4. Pieces of *Cuscuta trifolii* attached to the host initiate quick growth (A) and develop seeds after flowering (B).

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DIRECT AND RESIDUAL EFFECTS OF S AND Zn ON YIELD AND THEIR UPTAKE IN AN INDIAN MUSTARD (*BRASSICA JUNCEA* L.) – MAIZE (*ZEA MAYS* L.) CROPPING SYSTEM

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(Received: 19 May, 1998; accepted: 9 September, 1998)

Field experiments were conducted on a Fatehpur loamy sand (Typic ustochrepts) to study the effect of S and Zn application on Indian mustard and its residual effect on a subsequent maize crop. Indian mustard responded significantly to S and Zn applications. The highest grain yield was obtained when 20 kg S ha^{-1} was applied along with 11.0 kg Zn ha^{-1} with an increase of 45.3% over the control, while the application of S and Zn alone resulted in 30.6% and 22.7% increases in the yield. The oil content of mustard seed also increased by 2.0% after the combined application of S and Zn. A portion of the applied nutrients remained in the soil, significantly increasing the grain yield and the uptake of S and Zn in the subsequent maize crop. The residual effects of S and Zn should be considered when applying fertilizers to crops.

Key words: Indian mustard, maize, sulphur, zinc, direct and residual effects

Introduction

Several cereals and oilseed crops are known to respond to S (Ankineedu, 1983) and Zn (Kumar and Singh, 1979). Indian mustard (*Brassica juncea* L.) and maize (*Zea mays* L.) are important oilseed and cereal crops in northern India. The area under both crops has declined during the last two decades due to the dominance of the rice-wheat cropping system and their cultivation has been relegated to relatively coarse-textured soils which are prone to S and/or Zn deficiency. Both synergistic (Kumar and Singh, 1979; Sharma et al., 1990) and antagonistic (Shukla and Prasad, 1979) relationships have been reported between S and Zn. The interaction between these two nutrients deserves special attention for crop production, because zinc sulphate, commonly used to correct Zn deficiency in many crops, also adds sulphur to the soil.

The application of S and Zn benefits more than one crop in the sequence because of its residual effect on the subsequent crops, depending upon soil characteristics and the rate of application. There is a paucity of information on the direct and residual effects of these nutrients applied jointly as well as individually. The study was therefore undertaken to assess the direct effect of S and Zn on Indian mustard, their residual effect on the succeeding maize crop and the optimum S and Zn requirements in a mustard-maize cropping system.

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Materials and methods

A field experiment was conducted on Fatehpur loamy sand (Typic ustochrepts) deficient in zinc and sulphur at the experimental farm of Punjab Agricultural University, Ludhiana, India (latitude 30°56'N and longitude 75°32'E). The pH was 8.1, electrical conductivity 0.30 dS m^{-1} at 25°C (1:2 soil : water suspension), organic carbon 0.19% and the content of available N, P and K 160, 14 and 182 kg ha^{-1}, respectively. The soil contained 0.32 mg Zn kg^{-1} soil extractable by the DTPA method (Lindsay and Norvell, 1978) and 6.25 mg kg^{-1} S extractable with 0.15% $CaCl_2$ and determined according to Chesnin and Yien (1950). There were three rates of Zn (0, 5.5 and 11.0 kg ha^{-1} as zinc sulphate) except in the control plots, where zinc acetate was used, and four levels of S (0, 10, 20 and 40 kg ha^{-1} as gypsum) applied in all possible combinations to Indian mustard (1991–1992, cv. RLM-619). The residual effect of these treatments was studied on maize (cv. Partap). A basal dose of N, P and K was applied to Indian mustard at rates of 100, 13 and 25 kg ha^{-1} and to the maize crop at 120, 26 and 25 kg ha^{-1}. At 45 days of growth, samples were taken from the whole shoot for S contents and from the 1st, 2nd and 3rd most recently matured leaves for Zn content as per the procedure outlined by Harper and Berkenkamp (1975). The grain and straw/stover samples were taken at maturity. These samples were digested in a diacid mixture (4:1 HNO_3:$HClO_4$) and the digests were analysed for total Zn by atomic absorption spectrophotometry and for total S by the turbidimetric method (Chesnin and Yien, 1950). Organic carbon, pH and available P and K were determined by standard procedures (Page, 1982). The soil texture was determined by the hydrometer method (Sur and Singh, 1976). The oil content in the grains of Indian mustard was determined by wide line nuclear magnetic resonance.

Results and discussion

Direct effect on Indian mustard

The grain yield of Indian mustard increased significantly after both S and Zn application over the control (Table 1), but the magnitude of the response was much higher for S (31.5%) as compared to Zn (22.7%). This indicates that the

Table 1
Effect of S and Zn on the yield and S and Zn concentrations in Indian mustard

Treatment (kg ha^{-1})		Yield (t ha^{-1})		S concentration (%)			Zn concentration (μg g^{-1})		
Zn	S	Grain	Straw	Whole shoot	Straw	Grain	Index leaves	Straw	Grain
0	0	0.75	3.67	0.18	0.11	0.45	29.2	13.0	25.1
0	10	0.87	4.38	0.24	0.12	0.55	33.3	14.8	29.4
0	20	0.98	4.60	0.31	0.14	0.66	37.0	16.8	33.3
0	40	0.98	4.47	0.37	0.14	0.80	38.0	17.9	35.3
5.5	0	0.87	4.00	0.17	0.09	0.44	38.2	18.0	36.2
5.5	10	1.00	4.52	0.28	0.13	0.60	42.5	19.0	38.2
5.5	20	1.06	4.98	0.39	0.14	0.82	44.5	20.7	41.2
5.5	40	1.06	4.76	0.47	0.15	0.89	46.5	22.0	43.0
11.0	0	0.92	4.48	0.17	0.10	0.43	47.7	22.8	43.8
11.0	10	1.02	4.97	0.33	0.15	0.79	49.3	23.7	45.0
11.0	20	1.09	5.07	0.48	0.16	0.84	51.0	24.0	46.2
11.0	40	1.04	4.73	0.50	0.18	1.02	52.5	27.0	50.0
$LSD_{0.05}$		0.10	0.45	0.04	0.02	0.14	–	1.8	2.0

crop is more responsive to S than to Zn. This was expected, in view of the low content of both S and Zn in the soil. The maximum yield, however, was recorded when 20 kg S ha^{-1} was applied along with 11.0 kg Zn ha^{-1}. It is worth noting that the percentage response to applied S in the presence of Zn and to applied Zn in the presence of S decreased with their respective rates of application. This indicates that the application of one nutrient helps in the utilization of the other. The beneficial effect of the application of 20 kg S ha^{-1} along with 10 kg Zn ha^{-1} in increasing the mustard yield was also reported by Ankineedu et al. (1983). Tripathi et al. (1997) observed that the magnitude of the response in chickpea was more marked for S than for Zn addition.

A curvilinear relationship was obtained for grain yield when the levels of S were regressed at different rates of Zn. The regression equations calculated were as follows:

$Y_0 = 0.741+0.017x - 0.00027x^2$ (with 0 kg Zn ha^{-1})
$Y_1 = 0.872+0.014x - 0.00026x^2$ (with 5.5 kg Zn ha^{-1})
$Y_2 = 0.915+0.014x - 0.00028x^2$ (with 11.0 kg Zn ha^{-1})

where Y_0, Y_1 and Y_2 equals the grain yield in t ha^{-1} and x denotes the dose of S application.

The maximum response dose of S calculated from the above equations was found to be 31.5 kg ha^{-1} in the absence of Zn, decreasing to 28.5 and 26.0 kg ha^{-1}, respectively, in the presence of 5.5 and 11.0 kg Zn ha^{-1}. The lowering of the maximum response dose of S in the presence of Zn lends further support to the above observations. A similar trend was noticed for the straw yield.

S and Zn concentration and uptake

The concentration of S is used as an indicator of the S nutrition status of the plants. The concentration of S in the whole shoot, straw and grain in the absence of Zn application increased over the control treatment from 0.18 to 0.37, 0.11 to 0.14 and 0.45 to 0.80% at 40 kg S ha^{-1} (Table 2). The application of 5.5 kg Zn ha^{-1} significantly increased the S concentration in the whole shoot, straw and grain over the no Zn treatment at 10, 20 and 40 kg S ha^{-1}. Increasing the Zn application rate to 11.0 kg ha^{-1} further improved the S concentration in the plant, but the differences were non-significant. This further confirms that the application of Zn helps in the utilization of S by the plant. The grain yield had the highest coefficient of determination (R=0.74) with the S concentration in the whole shoot, followed by that in the grain (R=0.68) and straw (R=0.62). This suggested that the whole shoot (45 days growth) could be used as an indicator of the sulphur status of the plant. Using the statistical method of Cate and Nelson (1971) the threshold value of S concentration in the whole shoot was found to be 0.263%. The data revealed a lower grain yield when the S concentration in the whole shoot was below 0.263%, compared to cases when it exceeded this value. Bahl et al. (1986) reported the critical deficiency level of S to be 0.320 and 0.175% at 30 and 60 days of plant growth, respectively, in Indian mustard.

Table 2
Effect of S and Zn on total nutrient uptake and oil content in Indian mustard

Treatment (kg ha^{-1})		S content	Zn uptake	Oil content
Zn	S	(kg ha^{-1})	(g ha^{-1})	(% dry wt.)
0	0	7.3	66	41.0
0	10	9.9	91	41.9
0	20	12.7	110	42.6
0	40	13.9	115	42.8
5.5	0	7.4	104	41.6
5.5	10	12.0	124	42.0
5.5	20	15.6	146	42.8
5.5	40	15.9	150	43.0
11.0	0	9.3	143	41.6
11.0	10	15.5	164	42.2
11.0	20	17.2	172	43.0
11.0	40	18.8	178	43.0
$LSD_{0.05}$		1.8	14	0.6

There was an appreciable increase in the Zn content with the application of either Zn and S in all plant parts, but as the crop grew older the Zn content decreased, presumably due to a dilution effect. The combined application of Zn and S further improved the Zn status of the plant over their separate application and an almost similar pattern was recorded in all the plant parts. It is worth noting that the highest value of the coefficient of determination (R=0.66*) between recently matured leaves and the grain yield suggests that they could be used as an indicator plant part. The critical level of Zn in recently matured leaves, determined by the statistical method of Cate and Nelson (1971), was found to be 40 μg g^{-1}. Since no visual symptoms were observed on the leaves, values below this may be considered as low rather than deficient. This value also corroborates the lower yields obtained in treatments where the Zn concentration was below this value in the presence of an adequate amount of sulphur.

Sulphur uptake increased with the application of S and/or Zn, mainly due to an increase in the dry matter yield and the S content in the plants. The S uptake in the absence of Zn increased from 7.3 kg ha^{-1} in the control to 13.9 kg ha^{-1} after the application of 40 kg S ha^{-1}, whereas in the presence of 11.0 kg Zn ha^{-1}, S uptake increased from 9.3 kg ha^{-1} in the control to 18.8 kg ha^{-1} at 40 kg S ha^{-1} (Table 2). The zinc uptake by Indian mustard also increased with the application of Zn. The trend of Zn uptake due to the application of S and/or Zn was positive and significant, similar to that observed for S uptake. Similar results were reported for soybean (Kumar and Singh, 1979). Shukla and Prasad (1979), however, reported that S application decreased the Zn uptake in groundnut.

Oil content

The concentration of oil in mustard grain increased significantly over the control at all levels of applied S in the absence of Zn and at 20 kg S ha^{-1} and above in the presence of Zn. In the absence of S, Zn application did not materially influence the oil content of the seeds. The maximum increase of 2.0% in oil content was obtained when 20 kg S ha^{-1} was applied along with 11.0 kg Zn ha^{-1}, which was at par with the oil content obtained with 40 kg S and 11.0 kg Zn ha^{-1} (Table 2). The best grain yield was also obtained with 20 kg S and 11.0 kg Zn ha^{-1}. This indicates that in soils deficient in S and Zn, the application of 20 kg S and 11.0 kg Zn ha^{-1} may help in obtaining the potential yields and oil content in mustard. Aulakh et al. (1980) reported a 16% increase in the oil concentration of Indian mustard (*Brassica juncea* L.) with 60 kg S ha^{-1} on a similar soil.

Residual effect of S and Zn on maize

The application of Zn and S to Indian mustard has built up their respective available status in the soil to varying levels depending upon the rates applied. Zinc application raised the Zn status of the soil from the deficient to the sufficient level. These results also find support from the work of Takkar et al. (1975), who reported that application rates of 11, 22 and 33 kg Zn ha^{-1} could provide residual Zn for 5, 7 and 9 crops, respectively, in a wheat-groundnut cropping system. In the case of S, applied rates of 20 kg and 40 kg S ha^{-1} increased the available soil S level above the critical deficiency level, i.e. 10 mg S kg^{-1} soil. The grain yield of maize increased as the residual S in the soil increased, the difference in the residual level of S applied at 20 and 40 kg ha^{-1} being non-significant (Table 3).

Table 3
Residual effects of S and Zn on the yield and S and Zn concentrations in maize

Treatment (kg ha^{-1})		Yield (t ha^{-1})		S concentration (%)		Zn concentration (μg g^{-1})	
Zn	S	Grain	Stover	Grain	Stover	Grain	Stover
0	0	2.2	3.0	0.10	0.09	19.7	8.1
0	10	2.7	4.4	0.11	0.10	21.8	9.3
0	20	3.2	5.0	0.13	0.11	23.2	11.5
0	40	3.3	5.4	0.14	0.12	23.8	12.0
5.5	0	2.8	4.3	0.09	0.08	23.0	11.7
5.5	10	3.2	5.0	0.13	0.11	26.0	13.3
5.5	20	3.3	5.3	0.14	0.12	29.0	13.8
5.5	40	3.5	5.8	0.15	0.13	29.5	14.5
11.0	0	3.0	5.1	0.09	0.08	29.3	14.2
11.0	10	3.2	5.2	0.14	0.13	29.7	15.5
11.0	20	3.3	5.3	0.15	0.13	29.8	16.3
11.0	40	3.5	5.4	0.17	0.14	30.3	17.0
$LSD_{0.05}$		0.5	1.0	0.02	0.02	3.1	2.1

Pasricha and Aulakh (1986) also observed a significant residual effect on wheat when 20 kg S ha^{-1} was applied to groundnut. In a rice- mustard cropping system, the application of S and Zn is essential for efficient production and the added nutrients had a beneficial effect on the following crop, besides a possible increase in the nutrient status of the soil (Islam et al., 1997). In the absence of applied S, the maize grain yield increased due to the residual level of applied Zn, but the difference between the maize grain yield at 5.5 and 11.0 kg Zn ha^{-1} was non-significant.

The residual effect of the combined application of Zn and S improved the maize grain yield over the residual effect of single nutrients. In the presence of 5.5 kg Zn ha^{-1}, a significant increase in the maize yield was recorded due to the residual effect of 20 kg S ha^{-1}. This effect was statistically at par with that of the 40 kg S ha^{-1} treatment. In the presence of S, the residual effect of applied Zn only significantly increased the maize grain yield over the respective S levels up to 10 kg S ha^{-1}. The non-significant increase in the yield in Zn or no Zn treatments at higher residual S levels arose because of the efficient utilization of native Zn, which was marginal in the soil. Significant positive coefficients of correlation were observed between the grain yield of maize and residual S (r=0.82**) and Zn (r=0.61*) levels, indicating that available Zn or S, determined by DTPA and 0.15 $CaCl_2$, reflects the nutrient status of the plants and can be efficiently used for soil testing.

S and Zn concentration and uptake

The residual effects of increasing rates of S application significantly increased the S content in the maize grain from 0.10% in the control plot to 0.14% with carry-over S from the 40 kg S ha^{-1} treatment (Table 3). The carry-over effects of applied S increased the S concentration in the maize grain at all Zn rates except in the absence of Zn, where a slight decrease was noticed. Maximum S concentration in the grain (0.170%) was obtained when both nutrients were applied to the mustard crop at their highest rates of application.

Sulphur uptake by the maize crop continued to increase significantly with S and Zn application, mainly due to an increase in dry matter yield and sulphur content. It increased from 5.0 kg ha^{-1} in the control to 11.1 kg ha^{-1} after the application of 40 kg S ha^{-1}. The combined application of S and Zn had an additive influence and a maximum uptake of 13.5 kg S ha^{-1} was recorded at 40 kg S ha^{-1} and 11.0 kg Zn ha^{-1} (Table 4). A very similar pattern of Zn concentration and uptake was obtained in the grain and straw, the residual effects of both S and Zn helping to increase the total uptake by the crop. Total Zn uptake increased by 73 and 91 g ha^{-1} at 40 kg S ha^{-1} and 11.0 kg Zn ha^{-1}, respectively. After the cultivation of Indian mustard and maize, there was an adequate level of Zn and S in the soil with the application of 5.5 kg Zn ha^{-1} and 20 kg S ha^{-1}.

Table 4
Residual effects of S and Zn on their uptake in maize and on the available nutrient content in the soil after the maize harvest

Treatment (kg ha^{-1})		S uptake (kg ha^{-1})	Zn uptake (g ha^{-1})	Available nutrient status of the soil (mg kg^{-1})	
Zn	S			S	Zn
0	0	5.0	69	5.3	0.40
0	10	7.7	100	8.7	0.54
0	20	9.5	132	10.3	0.56
0	40	11.1	142	12.7	0.58
5.5	0	6.2	111	6.7	1.21
5.5	10	9.9	147	7.8	1.26
5.5	20	10.9	167	12.1	1.37
5.5	40	11.9	183	14.0	1.42
11.0	0	6.4	160	6.8	1.87
11.0	10	11.2	171	8.8	1.92
11.0	20	11.8	186	14.7	2.02
11.0	40	13.5	198	15.0	2.10
$LSD_{0.05}$		1.9	33	–	–

The above results indicated that a single application of 5.5 kg Zn ha^{-1} and 20 kg S ha^{-1} applied to the first crop of mustard is sufficient to obtain optimum yield in the succeeding maize crop.

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RESPONSES TO BORON IN PEARL MILLET AND CHICKPEA IN A POT EXPERIMENT WITH A NON-CALCAREOUS SOIL IN INDIA

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Boron deficiency may be a problem on high pH soil, but cereal crops often show symptoms of B toxicity when grown in semi-arid regions on alkaline soils containing inherently high levels of soluble B. Therefore, a greenhouse experiment was conducted in pots to observe the response to B in pearl millet (*Pennisetum typhoideum* L.) and chickpea (*Cicer arietinum* L.) in a non-calcareous soil. Chickpea was more sensitive to excess B than pearl millet. The magnitude of reduction in yield was greater in the grain than in the straw. The grain yield was reduced by 4.4% and 61% after the application of 0.8 and 2.0 mg B kg^{-1} soil, respectively. In the straw, a significant decrease in the yield was recorded at 0.8 mg B kg^{-1} soil. A gradual and consistent increase in B concentration was recorded in both the crops due to the application of B. The boron concentration in the straw was found to be higher than that in the grain in both the crops. The boron uptake in pearl millet increased up to the 1.0 mg B kg^{-1} soil level and decreased at 2.0 mg B kg^{-1} soil. In chickpea the B uptake increased up to the 0.8 mg B kg^{-1} level in the grain and up to the 0.4 mg B kg^{-1} soil level in the straw. The toxic effect of B was observed at 2.0 mg B kg^{-1} soil application in both the crops. The dry matter yield in chickpea was increased by a 0.2% foliar application of borax. The boron concentration and uptake in both the crops increased with B application.

Key words: boron, foliar spray, dry matter yield, B concentration, uptake of B

Introduction

Boron toxicity is a problem on about 90% of the salt-affected soils in India (Singh et al., 1995). Boron was found to be readily soluble in water (Phung et al., 1979), but its availability to plants decreased at higher pH. Availability also decreased sharply under drought conditions (Kalyaniratna et al., 1993). In coarse-textured soils low in available B the application of B in sorghum and pearl millet helped in increasing crop yield, while under similar conditions pulses were adversely affected. In general, the B contents of dicotyledonous plants are several orders of magnitude higher than those of monocotyledonous plants (Follet et al., 1981). Therefore, cereal crops often show symptoms of B toxicity when grown in alkaline soils of semi-arid regions containing inherently high levels of soluble B (Cartwright et al., 1984). Recently the foliar application of nutrients has gained considerable attention. The foliar application of boron has advantages over soil application, as it brings about direct translocation and an immediate improvement in the plants.

The present investigation was undertaken to study the effect of soil and foliar applications of B on the dry matter yield, concentration and uptake of B in pearl millet (*Pennisetum typhoideum* L.) and chickpea (*Cicer arietinum* L.) crops in non-calcareous soil.

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Materials and methods

Two experiments were conducted in the greenhouse to observe the response to boron in pearl millet and chickpea crops. A non-calcareous loamy sand soil (*Typic Ustipsamment*) was used, having pH 8.3, E.C. 0.15 dS m^{-1}, O. C. 0.30%, $CaCO_3$ - Nil, Sand 91.8%, Silt 5.7%, Clay 2.5%, available N, P, K 49.0, 4.2, 107.8 mg kg^{-1} soil, respectively, CEC 3.2 cmol $(P^+)kg^{-1}$ soil and hot water-soluble B 0.15 mg kg^{-1} soil. Five kg of air-dried soil (2 mm sieved) was filled into each polyethylene-lined earthen pot to avoid contamination. Each treatment was replicated thrice in a completely randomized design. Deionized water was used for watering the pots. Boron was applied at rates of 0, 0.25, 0.50, 1.00 and 2.00 mg kg^{-1} soil in the form of borax in the pearl millet experiment and 0, 0.1, 0.2, 0.4, 0.8, 1.6 and 2.0 mg kg^{-1} soil in the chickpea experiment. Foliar spraying with 0.1 and 0.2% borax (w/v) was carried out at the tillering stage, followed by two more applications at intervals of 10 days in both the crops.

A basal dose of N, P, K, Zn and S was applied at rates of 100, 50, 62.5, 5 and 20 mg kg^{-1} soil, respectively, in pearl millet and 25, 50, 62.5, 5 and 20 mg kg^{-1} in chickpea in the form of urea, KH_2PO_4, $ZnSO_4$ and $(NH_4)_2\ SO_4$. Pearl millet (HHB-67) and chickpea (H.N. 1) were raised as test crops and four plants per pot were maintained. The pearl millet crop was harvested before the emergence of the ears and chickpea at maturity. After washing and processing the plant samples were dried at 70 ± 2°C in a hot-air oven. The samples were ground in a stainless steel grinder and digested in a 4:1 diacid mixture of HNO_3:$HClO_4$, after which B was estimated by the azomethine-H method (John et al., 1975) using Spectronic-20.

Results and discussion

Visual symptoms

The crops did not show any deficiency symptoms to boron. However, stunted growth was observed in pearl millet at 2.0 mg B kg^{-1} soil and germination was delayed by one day due to boron toxicity. In chickpea, at 2.0 mg B kg^{-1} soil, germination was 100% but chlorosis appeared just after germination. On the 33rd day after sowing, toxicity symptoms appeared in chickpea at 0.8 mg B kg^{-1}. The plants were affected even at 0.4 mg B kg^{-1} soil, but only a slight response was observed with the 0.2% borax spray.

Dry matter yield

The application of B up to 1 mg kg^{-1} soil increased the dry matter yield of pearl millet significantly over its preceding level (Table 1). However, at 2 mg kg^{-1} soil the dry matter decreased in comparison to the 1 mg kg^{-1} soil level. The grain yield of chickpea was found to increase up to 0.2 mg B kg^{-1} soil (Table 2). After this level, the grain yield decreased and at 1.6 and 2.0 mg B kg^{-1} soil, severe toxic effects were observed. Similarly, the straw yield of chickpea increased up to 0.2 mg B kg^{-1} soil, but decreased beyond 0.8 mg kg^{-1} (Table 2). The results showed that chickpea is more sensitive to excess B than pearl millet. The magnitude of the reduction in yield was greater in the grain than in the straw. These results are in agreement with the findings of Singh et al. (1990) in wheat and Gupta et al. (1993) in chickpea and lentil.

Table 1
Effect of boron application on the dry matter yield of pearl millet at the ear emergence stage

B levels (mg kg^{-1} soil)	Dry matter yield (g pot^{-1})	Percentage response
0	12.50	–
0.25	13.30	6.45
0.50	15.50	24.00
1.00	22.70	81.60
2.00	19.80	58.40
$C.D._{0.05}$	2.00	–

Values are means of three replicates.

Table 2
Effect of boron application on the grain and straw yield of chickpea

B levels (mg kg^{-1} soil)	Yield (g pot^{-1})		Percentage response	
	Grain	Straw	Grain	Straw
0.0	15. 90	13.90	–	–
0.1	17.30	14.60	8.80	5.03
0.2	20.30	16.10	27.67	15.82
0.4	18.40	14.70	15.72	5.75
0.8	15.20	12.20	–4.40	–12.23
1.6	9.30	7.50	–41.51	–46.04
2.0	6.20	6.30	–61.01	–54.68
$C. D._{0.05}$	1.10	1.50		

Values are means of three replicates.

Boron concentration and B uptake in plants

In pearl millet straw the B concentration increased consistently from 40.8 mg kg^{-1} DM in control plants to 75.3 mg kg^{-1} DM in 2.0 mg B kg^{-1} soil treatments, whereas the uptake reached a maximum at the 1.0 mg B kg^{-1} soil level and started decreasing thereafter (Table 3). A significant increase was observed in the B concentration of both the grain and straw of chickpea with increasing levels of B in comparison to the previous level. The uptake of B by chickpea, however, increased significantly up to the 0.4 mg B kg^{-1} soil level and decreased thereafter both in grain and straw (Table 4). A gradual and consistent increase in the B concentration was recorded in both the crops due to the application of B. The boron concentration in the straw of both the crops was found to be higher than in the grain. Gupta et al. (1993) and Wong et al. (1996) also observed an increase in B content with applied B in chickpea and maize seedlings. The application of B resulted in a significant decrease in the uptake of B by wheat plants (Singh et al., 1990). This could be due to a decrease in the dry matter yield at higher levels of applied B.

Table 3
Effect of boron application on the B concentration and uptake of pearl millet

B levels (mg kg^{-1} soil)	B concentration (mg kg^{-1} DM)	B uptake (mg pot^{-1})
0	40.80	0.51
0.25	50.00	0.67
0.50	58.30	0.90
1.00	68.30	1.55
2.00	75.30	1.49
$C.D._{0.05}$	6.20	-

Values are means of three replicates.

Table 4
Effect of boron application on the B concentration and uptake of chickpea

B levels	B concentration (mg kg^{-1} DM)		B uptake (mg pot^{-1})	
(mg kg^{-1} soil)	Grain	Straw	Grain	Straw
0.0	15.42	54.17	0.24	0.75
0.1	17.92	59.17	0.31	0.86
0.2	19.58	63.75	0.40	1.03
0.4	22.92	76.83	0.42	1.13
0.8	27.50	86.25	0.42	1.05
1.6	31.67	119.58	0.29	0.90
2.0	55.83	137.08	0.35	0.86
$C. D._{0.05}$	3.20	15.50		

Values are means of three replicates.

Foliar application of boron

The dry matter yield of pearl millet increased after foliar spraying with 0.1% borax, but at the 0.2% borax level the yield decreased slightly as compared to 0.1% borax spray (Table 5). However, the dry matter yield of both the grain and straw of chickpea continued to increase up to the 0.2% borax level. The increase in the straw yield was higher than that of the grain at the 0.2% level, indicating that B supports more vegetative growth in chickpea at higher levels. The boron concentration and uptake increased in both the crops with an increase in the level of the foliar spray, but both B concentration and uptake were higher in chickpea than in pearl millet. Similar results were also observed by Rongfeng et al. (1975) in groundnut and Kalyaniratna et al. (1993) in pigeonpea crops. The 0.2% foliar application of borax was almost identical to the 2.0 mg B kg^{-1} soil application with regard to boron concentration and uptake in plants.

Conclusions

1. The dry matter yield of pearl millet increased significantly up to the 1.0 mg B kg^{-1} soil level, whereas the grain and straw yields of chickpea increased up to the 0.2 mg B kg^{-1} soil level over the control.

Table 5
Effect of foliar boron spray on the straw and grain yield, and on the concentration and uptake of B in pearl millet and chickpea crops

Foliar spray	Dry matter yield (g pot^{-1})	B concentration (mg kg^{-1} DM)	B uptake (mg pot^{-1})
	Pearl millet		
0	12.5	41.0	0.51
0.1% Borax	17.3	80.0	1.39
0.2% Borax	16.4	93.0	1.53
C.D. $_{0.05}$	2.0	6.0	–
	Chickpea		
0	13.9	54.0	0.75
	(15.9)*	(15.0)	(0.24)
0.1% Borax	14.8	135.0	1.99
	(18.2)	(46.0)	(0.84)
0.2% Borax	16.4	209.0	3.43
	(18.7)	(56.0)	(1.05)
C.D. $_{0.05}$	Straw 1.5	Straw 16.0	–
	grain 1.1	grain 3.0	–

*Values in parentheses denote grain.

2. Chickpea was found to be more sensitive to excess B than pearl millet.
3. The boron concentration in both the crops increased consistently and significantly at each level of B application in the soil over their preceding levels.
4. The maximum uptake of B in pearl millet was found at 1.0 mg B kg^{-1} soil level, while in chickpea it was at 0.4 mg B kg^{-1} soil.
5. After foliar spraying with borax the dry matter yield of pearl millet increased up to the 0.1% level, but in chickpea this increase was found up to the 0.2% level.

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WATER REGIME AND POROUS SYSTEM OF RED CLAYS IN HUNGARY

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Red clays in Hungary are the products of soil-forming processes taking place in earlier geological times. They are generally situated in areas that were mainlands in the Tertiary Period and so were not covered by marine sediments. Today they can only be found in areas where they were protected against degradation by ice in the Pleistocene or the material was able to withstand erosion. Their water regime and nutrient supply developed in the Holocene, but weathering and soil formation processes of earlier times also had a great influence on major properties. Today forests, vineyards and arable lands can be found on these red soils.

Key words: red clay, red soil, fossil soil, relict soil, soil water regime, pore space, differentiated porosity, water permeability

Introduction

Different ideas have developed on the formation of red clays in Hungary (Szabó, 1866; Lóczy, 1886; Ballenegger, 1917). According to these authors red clays have mostly been formed under a humid climate in tropical and subtropical areas and are relict or fossil soils. The formation processes of red clays were thoroughly studied by Kubiena (1958) who distinguished laterization, rubefication and the formation of "rotlehm" and "terra rossa". Stefanovits (1959, 1967) studied the genetics of red soils in Hungary. The composition and properties of red soils in Hungary have been investigated by several authors (Ötvös, 1954; Borsy and Szöőr, 1981; Jámbor, 1980; Jánossy, 1979; Kreetzoi, 1969; Pécsi, 1985; Schweitzer, 1993). Fekete et al. (1997) classified red clays in Hungary according to their mineral composition, distinguishing the following groups: the red soils of the Northern Borsod karst region, the bauxitic formations of the Transdanubian Hills, red soils formed on Permian sandstone and the soils of the border zone of the Great Hungarian Plain.

Tropical red ferrallitic soils are characterized by a relatively high caolinite clay mineral content and fairly high amounts of various ferric compounds (Fekete, 1988). These properties influence physical and water regime characteristics and may also determine the structure of the soil (Fekete, 1989; Klimes-Szmik, 1979). Against this background investigations were begun on the soil physics and water regime of red clays in Hungary. For these studies soil profiles of red clays with different mineral contents were chosen.

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Materials and methods

From among the numerous samples, soil profiles occurring on fairly large areas and having different mineral contents were selected for examination.

Soils examined:

1. Aggtelek Hills: Aggtelek – Béke-barlang, Vörös-tó, Jósvafő;
2. Cserehát Hills: Fancsal; foothills of the Mátra Hills: Visonta; Bakony Hills: Vörösberény; Szekszárd Hills: Kakasd II;
3. Bakony Hills: Hárskút, Padragkút, Szőc-1, Szőc-2; Szekszárd Hills: Kakasd I.

Basic pedological and mechanical analyses were performed using methods taken from Buzás (1988). Table 1 shows the results of basic soil science analyses, while the data of mechanical composition are presented in Table 2. The mineral composition of the soils was published in earlier studies (Fekete et al., 1997).

Table 1
Results of basic pedological analysis

Sample				K_A**	hy_1***	pH		$CaCO_3$	Humus
Site		No.	depth*			KCl	H_2O	(%)	(%)
			Aggtelek hills						
Aggtelek – Béke barlang		93.	0–15	47	3.74	5.40	6.34	0	3.67
		94.	15–30	46	3.95	5.50	6.29	0	4.29
		95.	40–70	44	4.18	5.68	6.62	0	1.83
Aggtelek –Vörös-tó		96.	0–15	54	4.60	4.84	6.24	0	0.82
		97.	25–50	49	4.72	4.80	6.06	0	
		98.	50–80	48	4.62	4.94	6.13	0	
Jósvafő		99.	0–20	55	5.75	5.91	6.57	0	3.36
		100.	20–55	62	9.72	4.74	5.92	0	0.19
		101.	55–85	54	5.57	4.91	6.14	0	
		102.	85–108	58	5.36	4.70	5.90	0	
			Fancsal – Visonta – Vörösberény – Kakasd II						
Fancsal	A_{11}	37.	0–10	46	4.03	6.81	6.26	0	2.32
	A_{12}	38.	10–30	48	4.79	5.22	5.90	0	1.13
	B_1	39.	30–52	48	4.79	4.39	5.88	0	0.02
	B_2	40.	52–82	46	4.87	4.21	5.83	0	
	B_3	41.	82–112	47	4.27	4.33	5.61	0	
Visonta		129.	120–140	60	4.34	7.66	8.63	1.63	0.62
Vörösberény	A_{sz}	64.	0–20	60	3.34	7.58	8.15	10.36	4.74
	A_1	65.	20–44	60	3.33	7.59	8.15	5.80	3.29
	B	66.	44–74	68	3.39	7.56	8.29	1.03	
Kakasd II		135.		52	6.08	7.67	8.24	4.49	2.43
			Bakony – Szekszárd hills						
Hárskút		53.	0–20	56	2.82	7.64	8.14	0.87	2.13
		54.	20–50	68	3.75	7.82	8.40	10.02	2.98
Padragkút	1.	56.		59	1.08	7.81	7.82	0.41	1.08
	2.	57.		69	0.93	8.03	8.04	0.12	0.38
Szőc-1.		132.	60–80	58	4.29	8.24	8.38	1.63	1.21
Szőc-2.		133.	200–220	56	1.64	7.90	8.35	4.90	1.44
Kakasd I		120.		51	3.73	7.74	8.36	0	0.18

*cm; ** K_A upper limit of plasticity according to Arany; *** hy_1 hygroscopicity according to Kuron

Table 2.
Mechanical composition (particle size distribution)

Sample				Percentage of particle fractions (%)							
Site		No.	Depth cm	>0.25	0.25–0.05	0.05–0.01	0.01–0.005	0.005–0.001	<0.001	>0.01	<0.01
						Aggtelek Hills					
Aggtelek Béke-barlang		93.	0–15	1.31	4.07	36.28	11.63	14.58	32.13	41.66	58.34
		94.	15–30	1.69	0.40	35.53	11.49	18.09	32.80	37.62	62.38
		95.	40–70	0.41	0.09	31.99	10.45	12.72	44.38	32.49	67.51
Aggtelek Vörös-tó		96.	0–15	0.91		24.52	5.20	12.86	56.51	25.43	74.57
		97.	25–50	0.12		22.27	7.13	8.19	62.29	22.39	77.61
		98.	50–80	0.36	0.18	23.76	4.17	11.83	59.70	24.30	75.70
Jósvafő		99.	0–20	0.16		22.73	7.79	12.83	56.49	22.89	77.11
		100.	20–55	0.64		20.17	6.76	13.29	59.14	20.81	79.19
		101.	55–85	0.02		24.66	5.28	10.40	59.64	24.68	75.32
		102.	85–108	0.06	1.84	.24.82	0.97	10.09	62.22	26.72	73.28
						Fancsal-Visonta-Vörösberény-Kakasd II					
Fancsal	A_{11}	37.	0–10	2.34	6.82	38.75	0	30.46	21.63	47.91	52.09
	A_{12}	38.	10–30	2.34	3.47	28.70	7.48	14.20	43.81	34.51	65.49
	B_1	39.	30–52	3.13	8.72	25.48	4.55	15.42	42.70	37.33	62.67
	B_2	40.	52–82	2.96	6.54	27.14	9.55	8.82	44.99	36.64	63.36
	B_3	41.	82–112	3.74.	8.50	28.05	7.51	12.22	39.98	40.29	59.71
Visonta		129.	120–140	1.68	5.62	18.15	2.91	6.66	64.98	25.45	74.55
Vörösberény	A_{sz}	64.	0–20	2.04	10.72	23.90	4.13	12.56	46.65	36.66	63.34
	B_1	65.	20–44	3.41	9.85	19.09	5.65	21.90	40.09	32.35	67.65
	B_2	66.	44–74	5.65	2.98	20.47	2.09	11.82	56.99	29.10	70.90
Kakasd II		135.		2.29	2.13	36.77	5.43	10.34	43.04	41.19	58.81
						Bakony Hills-Szekszárd Hills					
Hárskút		53.	0–20	0.27	9.55	40.47	8.60	14.28	26.80	50.30	49.69
		54.	20–50	0.49	1.87	22.52	6.66	13.77	54.69	24.88	75.12
Padragkút		56.		3.24	1.84	7.53	7.39	38.99	41.01	12.61	87.39
Szőc-1.		132.	60–80	14.00	12.98	7.88	6.07	26.36	32.71	34.86	65.14
Szőc-2.		133.	200–220	4.81	11.27	7.46	3.81	11.26	61.39	23.54	76.46
Kakasd I		120.		1.17	41.10	5.48	1.57	3.89	46.79	47.75	

Results

Different moisture forms and differentiated pore space values of red clays

Figures 1–9 show the data of different forms of moisture, total pore space and differentiated porosity.

Figures 1–2 summarize the results of the analysis of soils from the Aggtelek Hills. Values of unavailable water were calculated from hygroscopic moisture content values according to Klimes-Szmik (1957, 1962). The calculated amount of unavailable water increased in the order Béke-barlang, Vörös-tó, Jósvafő. This was related to the clay content. The amount of clay in the profile near Béke-barlang was 58–67%, in that near Vörös-tó 74–77%, and in that in the Jósvafő profile around 75–79%. The percentage of particles smaller than 0.001 mm was 32–44%, 56–59% and 56–62%, respectively. The values of the sticky point according to Arany and the hy_1 values increased in the same order (Table 1).

The different water capacity values were the highest in the profile near Jósvafő (Fig. 1), where the sticky point according to Arany and the hygroscopic values were also high. The lowest water capacity and unavailable water values were found in the Vörös-tó profile, probably due to the high amount of iron and manganese coating. The available water content (minimal water capacity – unavailable water) was at the same level in all three profiles.

The distribution of pores of different quality and size was characterized by a high percentage of medium size pores (capillary pores and the pore space of entrapped air). The pore space of the entrapped air was considered as being of medium size, assuming that air remained in them in the case of capillary saturation. The space of fine pores (pore space of strongly and loosely absorbed water) and the space of coarse pores (total gravitational pore space and gravitational-capillary pore space) were relatively small. The distribution of fine (micro), medium size and coarse (macro) pores approximated to the ideal 1:1:1 proportion in the lower layer of the profiles near Béke-barlang and in the upper layer of the Vörös-tó and Jósvafő profiles.

All other soil profiles were found in different parts of the country, but two groups could be distinguished on the basis of similar moisture content values and differentiated porosity.

The Fancsal, Visonta, Vörösberény and Kakasd II profiles belonged to the first group, while the red clays with bauxitic formations from the Bakony Hills and the Kakasd I soil profile belonged to the second.

The common characteristics of the Fancsal, Visonta and Kakasd II profiles were the high unavailable water content and the medium moisture content at minimal water capacity moisture content (Fig. 3). Owing to its similar properties the Vörösberény profile with bauxitic formations was also included in this group. The high amount of unavailable water and the water capacity value were due to the higher clay content. The percentage of particles smaller than 0.01 mm varied between 40–60% (Table 2). The hygroscopic moisture content of these profiles was also high. Within the clay content the amount of

montmorillonite, which has a high specific surface area, was considerable, especially in profiles from Fancsal and Visonta. The soil from Visonta had a high clay content; the percentage of particles smaller than 0.001 mm was 65%. This explained the fact that the hygroscopic water content, unavailable water and minimal water capacity values were outstanding (Table 1, Fig. 3).

In the profiles of this group the ratios of fine, medium size and coarse pores were almost equal, the only exception being the Visonta sample, in which the ratio of coarse pores was very low (Fig. 4).

Of the water regime characteristics of the Hárskút, Padragkút and Szőc profiles from the Bakony Hills and the Kakasd I profile from the Szekszárd Hills, the minimal water capacity values were quite low (Fig. 5).

The hygroscopic water contents and unavailable water contents of these profiles were low except for the Kakasd I profile. Hence their available water contents (minimal water capacity – unavailable water) were high. These properties were the result of the relatively high clay content and caolinite clay mineral content. The distribution of pores of different size was characterized by a high proportion of medium size pore spaces. While the ideal ratio is 1:1:1, the proportion of the medium size pore spaces varied between 1.9–2.6 and the ratio of fine and coarse pores was very low (Fig. 6).

When measuring pore spaces the expansion and contraction of the soils caused a certain amount of difficulty. In several cases the volume of samples taken with the Klimes-Szmik sampling cylinders decreased considerably during drying. The moisture content was very high when the samples were taken, in some cases approaching the minimal water capacity values. From the decrease during drying it follows that at lower moisture content the particle volume of the sample was higher and the total pore space lower. Therefore, the degree of contraction must be considered when examining differentiated porosity and water permeability.

On the basis of the degree of expansion and contraction two groups could be distinguished among the soils investigated. Soils characterized by montmorillonite clay mineral contents of around 40% or higher belonged to the first group. These included the samples from Fancsal, Aggtelek (Béke-barlang), Jósvafő and Visonta and the Kakasd I and II samples. In these samples the difference between volume density and calculated pore space was considerable. The difference between the pore space of wet and dried soil was around 7–10%; in the upper layer of the Jósvafő sample it was 14%.

In the soils of the second group the dominant clay mineral was caolinite. Samples from Vörösberény, Hárskút, Padragkút and Szőc belonged in this category, with caolinite contents of 32–39% and high boehmite, gibbsite, hematite and goethite contents. In these soils the above-mentioned differences were much smaller, the pore spaces of wet and dried soils varying by only 1.5–6.6%.

The existence and extent of expansion and contraction influence several water regime properties of the soil. For example, in the soils of the first group the hygroscopic water content, the unavailable water content, the sticky point

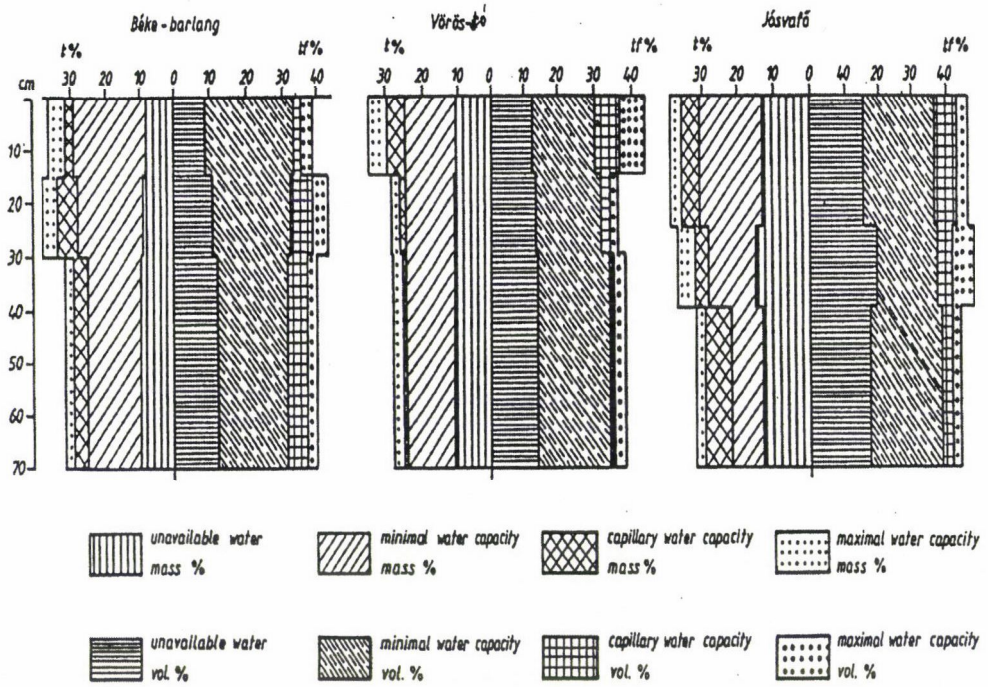

Fig. 1. Values of unavailable water and water capacity. Béke-barlang, Vöröstó, Jósvafő

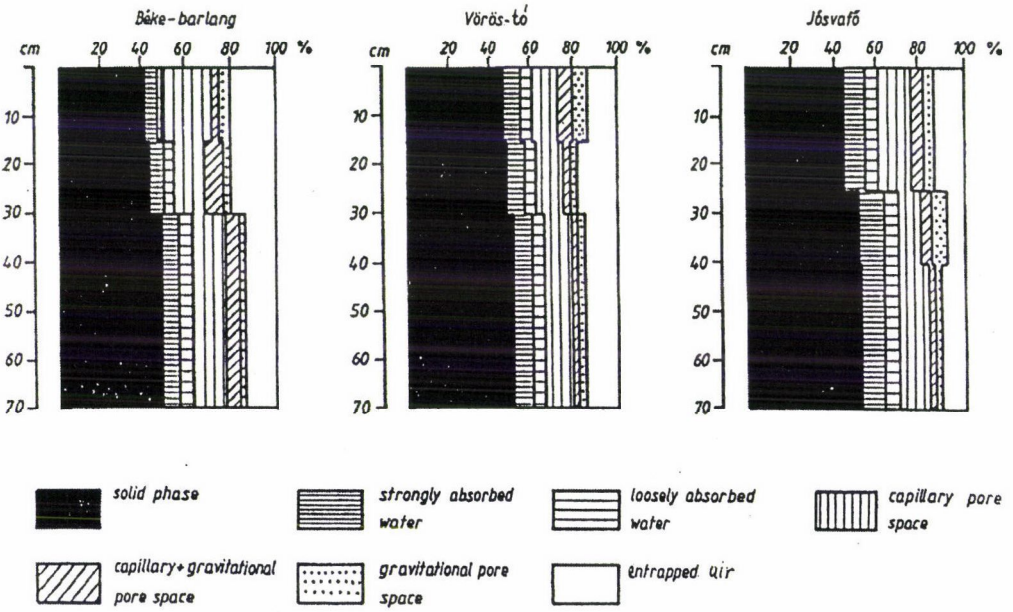

Fig. 2. Differentiated porosity. Béke-barlang, Vöröstó, Jósvafő

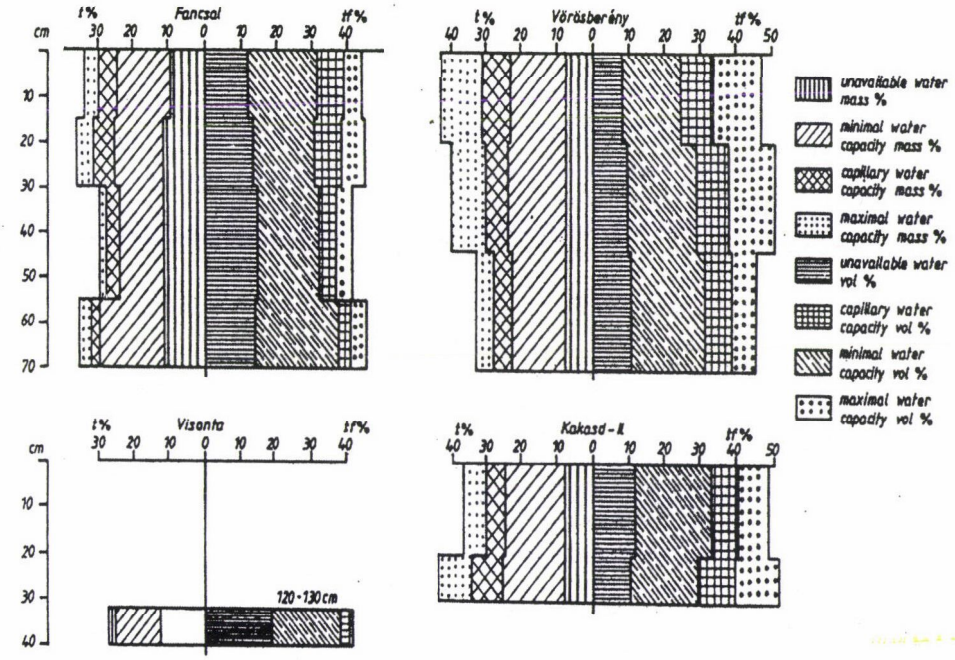

Fig. 3. Values of unavailable water and water capacity. Fancsal, Vörösberény, Visonta, Kakasd-II

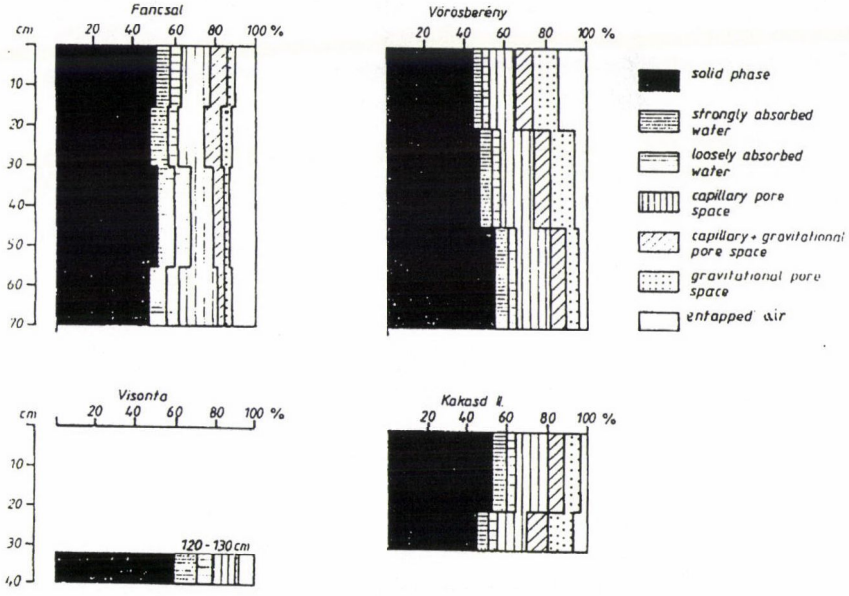

Fig. 4. Differentiated porosity. Fancsal, Vörösberény, Visonta, Kakasd II

according to Arany (Fig. 1) and the pore space of strongly and loosely absorbed water were higher (Figs 1–6). These soils were characterized by a sudden decrease in the rate of water permeability during the first hours (Figs 7–8).

The most conspicuous characteristic of the second group was the large capillary pore space, while the rate of water permeability was almost uniform, varying only slightly in time.

Tensions brought about by the expansion and contraction of the soils also played a role in the formation of the soil structure. This was shown by profiles without plant cover sampled near Aggtelek and Jósvafő.

The conclusions drawn during studies on the different moisture forms and differentiated porosity can be summarized as follows:

- Differences between soils characterized by nearly the same mineral composition were related to the clay content. The hygroscopic water content, the volume of absorbed water and the different water capacity values were higher if the clay content was higher.
- Besides clay content the different mineral compositions of the soils played an important role in the formation of the water regime properties of the soil.
- In soils with higher montmorillonite contents the hygroscopic value, the sticky point and the water capacity value were also higher. The distribution of fine, medium size and coarse (macro) pores was more uniform than in any other group of soils.
- Red clays containing the clay mineral caolinite and characterized by bauxitic mineral composition contained lower quantities of hygroscopic moisture and absorbed water. From among the different size pores, medium size pores were dominant, while the ratio of fine and coarse pores was low.
- Red clays that contained higher amounts of montmorillonite expanded considerably when they were saturated with water and contracted when they were dried. The difference between the volume density and pore space of wet and dry soil was substantial. The difference in pore space was about 7–10%.
- The difference between the pore space of wet and dried clays containing caolinite and/or bauxite was only 2–4%.

Water permeability

The water permeability of the original structure of some of the samples was determined. The investigations were carried out in a laboratory according to Klimes–Szmik (1962). Figures 7–8 show the water permeability values and the rate at which the water permeability changed.

Aggtelek Hills

The highest water permeability values were found in the upper layers of the profile near Béke-barlang. In the upper layers of the profile the amount of water filtering through during 5 hours was 100–200 ml/cm^2. The rate of water permeability was also relatively high, being 0.8–1.4 ml/cm^2/min in the first hour

and 0.5–0.8 ml/cm^2/min in the fifth. In the layer at a depth of 40–50 cm these values were lower. The water permeability of the soil near Vöröstó was much smaller, being 32–55 ml/cm^2 after 5 hours, while the rate of water permeability was only 0.15–0.25 ml/cm^2/min. The soil near Jósvafő had the smallest water permeability; even in the upper layer it was only 20–22 ml/cm^2, with a rate of 0.12–0.13 ml/cm^2/min.

The water permeability of the three soil profiles was proportional to the pore space conditions (Figs 2–3). The total pore space was highest in the Béke-barlang samples, lower in the Vörös-tó samples and the lowest in the Jósvafő samples. The water permeability data showed a similar tendency. Water permeability was also correlated with the ratio of coarse (macro) pores. The higher water permeability of the soils near Béke-barlang was related to better structure and a dense root system. (The sample from Béke-barlang originated from under a grass community in a sparse forest. The soil could be easily crushed and had a polyhedral structure densely interlaced by roots.) Water permeability correlated best with the total number of medium and coarse pore spaces. The water permeability was the highest where the total of capillary, capillary-gravitational and gravitational pore spaces was the highest. On the other hand, the pore space of strongly and loosely absorbed water correlated reciprocally with water permeability.

There was also a relationship between water permeability, soil mechanical composition (Table 2) and the plasticity of the soil (Table 1). The higher the amount of clay, the lower the water permeability was. Water permeability showed a mutual correlation with the number of particles smaller than 0.01 mm and the percentage of the loess fraction. The soil near Béke-barlang had the highest loess fraction, where the highest values of water permeability were found.

No correlation could be shown between water permeability and mineral composition, but it should be noted that in the soils of Vörös-tó and Jósvafő there was much more red iron coating and black manganese coating. This influenced the water permeability only through its impact on soil structure and porosity.

Fancsal, Vörösberény, Kakasd

All three profiles were characterized by low water permeability values. The water permeability of the soils from Fancsal became slower after an initial fast water permeability, and the values after 5 hours were the lowest in this profile. Water permeability was low in the other two profiles as well, the rate of water permeability being fairly uniform, hardly varying in time. Out of the three profiles the sample from Vörösberény had the best water permeability and the sample from Fancsal the worst (Fig. 8).

The somewhat better water permeability of the Vörösberény soil showed a direct correlation to the higher ratio of coarse pores. By comparing water permeability with the results of pedological analyses conclusions could be drawn regarding the role of the clay content, quality and mineral composition. Soils with higher caolinite and lower montmorillonite contents had better water

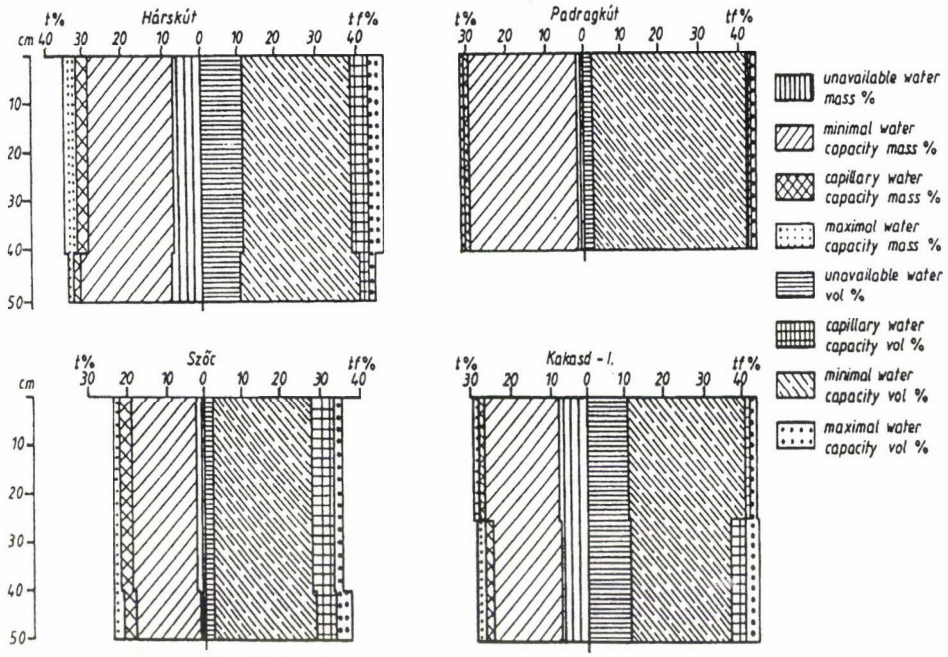

Fig. 5. Values of unavailable water and water capacity. Hárskút, Padragkút, Szőc, Kakasd I

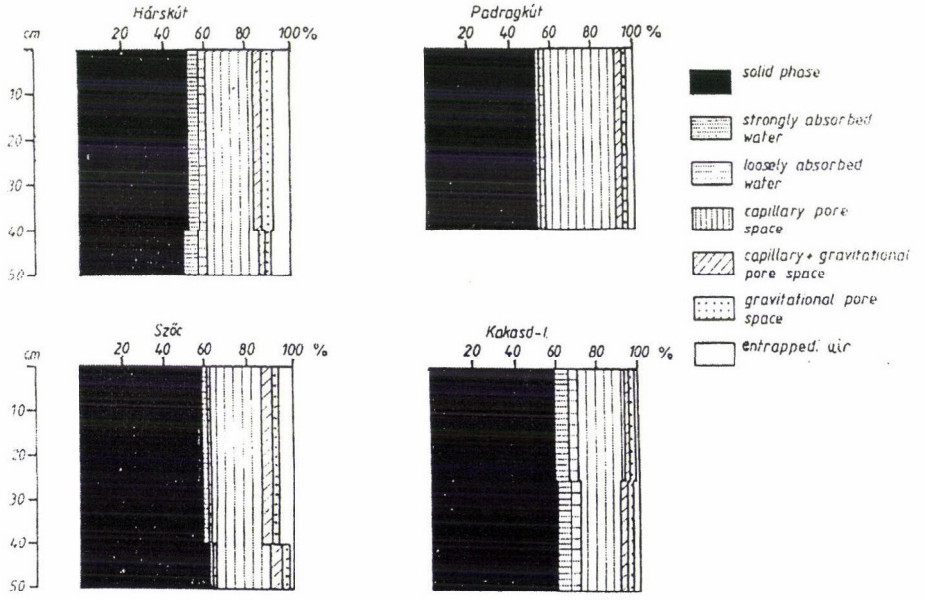

Fig. 6. Differentiated porosity. Hárskút, Padragkút, Szőc, Kakasd I

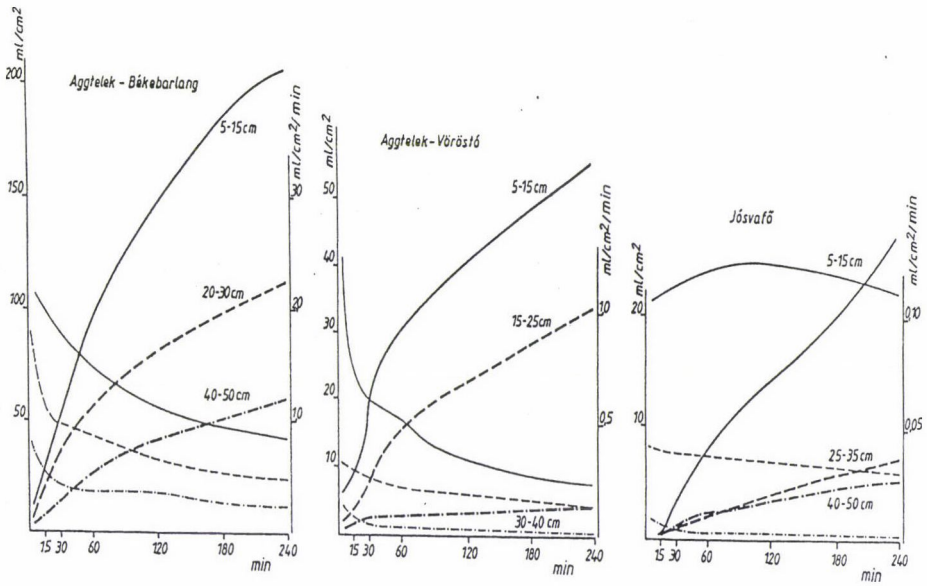

Fig. 7. Water permeability curves. Béke-barlang, Vöröstó, Jósvafő

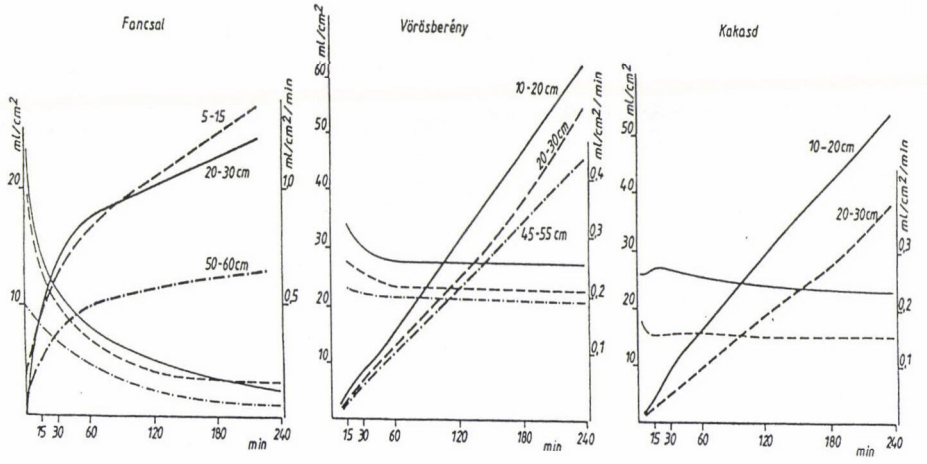

Fig. 8. Water permeability curves. Fancsal, Vörösberény, Kakasd

permeability. Samples from Vörösberény had a fairly high clay content; their montmorillonite content was the lowest, but their caolinite content was the highest, amounting to 32–35%. In the other two profiles montmorillonite occurred in much higher amounts and there was much less caolinite. The expanded montmorillonite clay mineral made the water permeability of the soil lower. Obviously the substantial decrease in the initially high water permeability of the soil samples from Fancsal can be explained by the expansion of the montmorillonite. The montmorillonite content of this profile was 37–42%.

The water permeability of soil containing the non-bulging caolinite clay mineral was better and the rate of water permeability was relatively uniform. In these samples the distribution of the different pore spaces was also more favourable, the ratio of smaller pores was lower and the ratio of medium size pores was relatively high (Figs 2–3).

The following conclusions can be drawn from the studies on the water permeability of the soils:

- Water permeability is correlated with the pore space and the ratio of coarse pores. The bigger the total pore space and the ratio of coarse pores, the better the water permeability of the soil.
- If the clay content is higher the water permeability is generally lower.
- In soils that contain montmorillonite the speed of water permeability is lower than in soils that contain caolinite. Within the porous system of bauxitic red clays containing caolinite the volume of medium size and coarse pores increases, resulting in better water permeability.
- In the upper layers of soils covered with vegetation water permeability is much better. The high amount of roots and root residues provides better soil structure and porosity, thus improving water permeability.

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INTERACTION BETWEEN FERTILIZATION AND SOIL CULTIVATION IN MAIZE PRODUCTION

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The effect of crop production factors on the maize yield was studied on a chernozem soil with lime deposits at the Látókép Experimental Farm of the Department of Crop Science and Land Use of Debrecen Agricultural University between 1989 and 1994. A multifactorial long-term experiment made it possible to evaluate the effects and interactions of fertilization and soil cultivation. Soil cultivation can only be considered to be up-to-date and adaptable if it is adjusted both to soil status and to farming conditions. The method, tool and depth of cultivation must be chosen to suit the physical state of the soil. A consideration of the moisture content of the soil reduces damage to the soil structure.

The experimental results prove that on a chernozem soil, provided precipitation supplies are at least average, winter ploughing, even without fertilization or with low fertilizer rates, causes a substantial increase in the maize yield by making the soil nutrients available to the plant. The extra yield achieved with winter ploughing without fertilization was 1.4–2.3 t/ha compared to the unploughed variant. In fertilized treatments the yield increase was greater, averaging 2.8–3.3 t/ha. In agreement with other findings, spring ploughing resulted in an unfavourable soil state, retarding germination and hindering uniform, rapid emergence, a fact which was also reflected in the yield. Over a six-year average, the yields were 0.6 t/ha lower after spring ploughing than after winter ploughing. In dry years the yield difference was somewhat smaller, but under favourable rainfall conditions maize grown after spring ploughing yielded 1.0–1.4 t/ha less than after winter ploughing.

Fertilization was able to compensate in part, but not entirely, for other unfavourable agrotechnical effects. Without ploughing the extra yield due to fertilization was 2.8 t/ha in dry years and 3.8 t/ha under more favourable rainfall conditions. After winter ploughing the difference between the years was smaller (0.6 t/ha), with an extra yield of 3.1 t/ha in dry years and 3.7 t/ha after average rainfall. The yield-increasing effect of fertilization, averaged over the six years examined, differed in the three soil cultivation variants. Linear and quadratic terms were significant in the fertilizer effect, so the relationship can be described well using an optimum curve. The breakdown of the interaction between fertilization and soil cultivation using orthogonal polynomes indicates that in the linear phase, representing initial low fertilizer rates, there is no substantial difference between the three types of soil cultivation. In optimum fertilizer treatments, however, the differences in yield increase were considerable for all three soil cultivation treatments and the interaction between the quadratic effect of fertilization and soil cultivation was significant.

Considerable savings in time, energy and costs can be achieved through the better exploitation of agronomic factors. When planning fertilization and cultivation methods, attention should be paid to regional potential, ecological conditions, including the state of the soil when cultivated, factors determined by the forecrop and the requirements of the crop to be grown.

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Introduction

Relying on the findings of a number of years Győrffy (1966) found that on soil with good fertility maize responds not so much to direct fertilization as rather to the general level of nutrient supply in the soil. In a similar way, Surányi (1957), Bauer (1959) and Dezső and Martin (1965) emphasized that it is more effective to plan and implement fertilization within the framework of the whole crop rotation.

According to Bocz (1976) water supply plays a significant role in the utilization of the active agents of fertilizers, especially nitrogen. Hungarian and foreign publications alike agree that weather is one determinant of the factors influencing the fertilizer effect. Since the weather regulates the heat and water supplies of the growing area, it has an effect on material transformation in the soil, on the growth, nutrient uptake and yield of crops, and therefore on the utilization of fertilizers (Hank and Frank, 1951; Szász, 1988; Kovács, 1982; Jolánkai, 1982; Nagy, 1988; Ángyán, 1991; Kádár, 1992; Varga Haszonits and Mikéné Hegedűs, 1993; Hall et al., 1994). The amount of precipitation and the water reserves in the soil modify both the fertilizer requirement and the fertilizer effect. As the optimum water supply is approached the fertilizer effect increases, after which, when the point of adverse water surplus is reached, it decrease again (Harmati, 1987). The influence of the soil characteristics depends primarily on the soil fertility, the depth of the surface soil and the water balance (Sarkadi, 1975; Buzás, 1987; Németh and Buzás, 1991; Ruzsányi, 1992).

When studying the relationship between the depth of soil cultivation and fertilization Sipos (1968, 1974) found a positive correlation in the case of maize. Of the two factors the effect of fertilization is decisive. Dry years modify the interaction of the two elements. Kemenesy (1972) pointed out that after radical deep ploughing the effect of the same fertilizer dosage is smaller than after medium-deep ploughing. This is partly due to the fact that in the deep soil layers the active agent of the fertilizer will be diluted and the ploughed-up subsoil is poor in nutrients. This cannot be compensated for even by the favourable physical influence (porosity improvement) of deep soil cultivation. Kovács (1964) carried out investigations to evaluate the relationship between tillage and soil moisture changes. He found that the depth of ploughing and the soil moisture content were in correlation down to a soil depth of 150 cm. Studies on the effect of tillage on the soil and the crop yields prove that it is worth considering a soil cultivation system covering several years rather than the results of single years (Sipos, 1958; Nyíri, 1973; Wildman and Gowans, 1975; Győrffy, 1977; Neigi, 1982; Birkás et al., 1989; Kapocsi, 1984; Birkás and Szabó, 1992). The physical degradation of the soils is increased by the packing effect of big, heavy farm machines and excessive tillage operations. The soils become more compact and their water balance (water drainage and water holding capacity) declines (Stefanovits, 1975; Boels, 1982; Dickson, 1983). Therefore, the application of minimum tillage methods and tools are desirable (Birkás, 1987, 1993; Barta and Jóri, 1979; Sörös and Soós, 1994). However,

during the assessment of the effects of reduced tillage methods, not only must the soil structure be taken into account, but also changes in soil moisture and heat circulation need to be investigated as well as nutrient mobilization. These elements frequently alter in an unfavourable direction (Massee, 1982). According to Herbert (1982) soil deterioration is caused by a number of processes. The lack of fertilizer application, a monoculture or inadequate crop rotation may all contribute to soil compaction, thus reducing soil fertility and increasing cultivation expenses. Stefanovits (1975) and Várallyai (1976, 1978) drew attention to the same problem when insisting on the redefinition and enlargement of the tasks of soil sciences in agricultural production development. Of the foreign specialists, Doran (1982) lays particular stress on the importance of the biological impact of soil cultivation.

Materials and methods

At the Látókép Experimental Farm of the Department of Crop Science and Land Use of Debrecen Agricultural University the effects of crop cultivation factors on the yield of maize have been studied on a chernozem soil with lime deposits since 1980.

A multifactorial long-term experiment allows for the evaluation of the effects of fertilization and soil cultivation. The major block was subdivided for each tillage method and each hybrid. In all, fertilization treatments were set up in a randomised block design with four replications. The maize hybrids examined were Volga SC, Pannonia SC and Dekalb 524 SC. The size of each tillage block was 4,320 m^2 and the size of each fertilization plot was 360 m^2 (3 × 360 = 1,080 m^2).

The treatments were as follows:

Fertilization	M_1 = without fertilization (control)
	M_2 = 120 N + 90 P_2O_5 + 106 K_2O kg/ha
	M_3 = 240 N + 180 P_2O_5 + 212 K_2O kg/ha
Tillage	T_1 = without ploughing (12 cm)
	T_2 = spring ploughing (22 cm)
	T_3 = winter ploughing (27 cm)

Soil characteristics

The soil of the Experimental Farm is chernozem with lime deposits developed on loess soil. It has moderate supplies of N and P and a high K content (humus content = 2.8–3.0%, total N = 0.14–0.18%, AL-P_2O_5 = 130–200 mg/kg, AL-K_2O = 240–280 mg/kg). The depth of the humus layer is 70–90 cm. The pH value (KCl) is 6.2; the "Arany"-type liquid limit is 43. Microelement deficiency cannot be detected. The groundwater level is between 6–8 m. The minimum water holding capacity (WHC_{min}) of the soil is 27–29 volume %. The water storage capacity of the soil is 275 mm in the 0–100 cm profile and 265 mm in the 100–200 cm profile. The available field capacity (FC) is 157 mm and 150 mm in the 0–100 and 100–200 cm profile, respectively.

Weather characteristics

In Debrecen, over the six years studied, the precipitation was unfavourable (drought) to maize in 1990, 1992 and 1994, while in 1989, 1991 and 1993 the rainfall conditions were average.

In order to ensure the reliability of the evaluation up-to-date experimental design methods were applied in the planning of the research project, using an improved version of the method reported by Box and Wilson (1951). In the evaluation of the experimental data variance analysis was used with the disaggregation of the variance components (Sváb, 1981; John, 1971; Winer, 1971). During the analysis all the treatments set up in the experiment were considered according to their design. Thus, in the model of variance analysis the tillage methods, applied in the main plot,

were regarded as group factors and the fertilization treatments, set up in the subplots, were taken as trial factors (Huzsvai, 1994). This model was a fixed, repeated measuring model. The effect of fertilization was broken down by means of orthogonal polynomes. Linear and quadratic effects were distinguished. One advantage of the orthogonal polynomes is that they are completely independent of each other, which means that no correction is needed for the variances when estimating significance. Another advantage is that they can be used to produce univariant components, so the presumption of symmetry can be neglected. The effects and primary interactions of each element (soil cultivation, fertilization) in the multifactorial experiment were studied over the average of the other treatments.

The evaluation was done on an IBM 486 DX computer with the 1988 version of the BMDP Statistical Software.

Results and discussion

The results of the fertilization and tillage research conducted at the Látókép Experimental Farm of the Department of Crop Science and Land Use of Debrecen Agricultural University are in agreement with the results of similar long-term experiments carried out in Martonvásár. The experimental data were evaluated yearly and over the whole of the period studied, using the method of variance analysis. During the analysis all the treatments set up in the experiment were considered according to their design.

Regarding the results of the variance analysis, over the six years studied, excluding 1990, the effect of tillage on the yields was significant in each year and also over the average of the six years examined. In all five years, the yields after winter ploughing differed significantly both from those achieved without ploughing and from those after spring ploughing. Considering the $LSD_{5\%}$ values, in the year 1992 and on the six-year average there was no significant difference between the harvest data achieved without ploughing and the yields after spring ploughing.

In crop production, soil cultivation has achieved its aim if the seed-bed is adequate and provides good conditions for the rapid germination and emergence of maize and for the development of a uniform crop stand. Over the average of six years, including three droughty years and three with an average precipitation supply, winter ploughing provided the best conditions for maize; the yield was 1 t/ha (12%) more than that achieved without ploughing (Fig. 1). In dry years the favourable influence of winter ploughing was less (max. 0.5 t/ha).

The experimental results prove that on a chernozem soil, provided precipitation supplies are at least average, winter ploughing, even without fertilization or with low fertilizer rates, causes a substantial increase in the maize yield by making the soil nutrients available to the plant. The extra yield achieved with winter ploughing without fertilization was 1.4–2.3 t/ha compared to the unploughed variant. In fertilized treatments the yield increase was greater, averaging 2.8–3.3 t/ha.

Considering the results of the variance analysis, over the six years studied the effect of fertilization on the yields was significant in each year and also over the average of the six years examined.

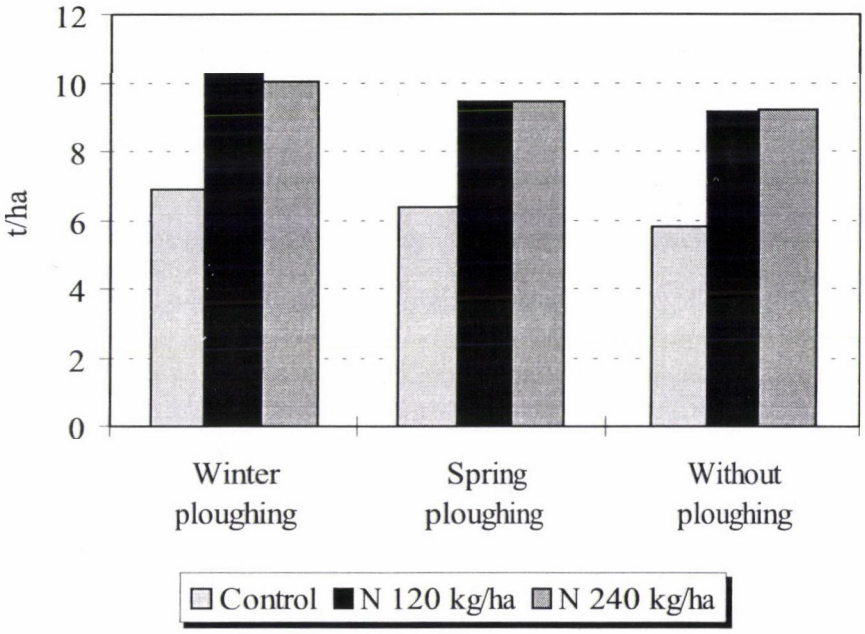

Fig. 1. Effect of fertilization and soil cultivation on maize yields. Debrecen, average of the years 1989–1994

Except for the year 1990, the relationship between soil cultivation and fertilization was significant (Table 1). Fertilization was able to compensate in part, but not entirely, for other unfavourable agrotechnical effects. Without ploughing the extra yield due to fertilization was 2.8 t/ha in dry years and one ton per hectare more (3.8 t/ha) under more favourable rainfall conditions. After winter ploughing the difference between the years was smaller (0.6 t/ha), with a substantial extra yield of 3.1 t/ha in dry years and 3.7 t/ha after average precipitation.

In agreement with other findings, spring ploughing resulted in an unfavourable soil state, retarding germination and hindering uniform, rapid emergence, a fact which was also reflected in the yields. Over a six-year average, the yields were 0.6 t/ha lower after spring ploughing than after winter ploughing. In dry years the yield difference was somewhat smaller, but under favourable rainfall conditions maize grown after spring ploughing yielded 1.0–1.4 t/ha less than after winter ploughing. Depending on the weather conditions in different years, the extra yield caused by fertilization was 2.8–3.2 t/ha compared to the treatments without fertilization.

Table 1
Effect of fertilization and soil cultivation on maize yields. Debrecen, 1989–1994

Tillage	Fertilization	Yield, t/ha						Mean
		1989	1990	1991	1992	1993	1994	
Winter ploughing	Control	8.74	7.63	9.04	4.88	6.00	4.98	*6.88*
	120 kg N/ha	12.51	10.09	12.09	7.97	10.37	8.59	*10.27*
	240 kg N/ha	12.86	9.91	11.99	7.00	10.22	8.07	*10.01*
	Mean	***11.37***	***9.21***	***11.04***	***6.62***	***8.86***	***7.21***	***9.05***
Spring ploughing	Control	7.78	7.62	9.53	4.11	4.97	4.28	*6.38*
	120 kg N/ha	11.42	10.11	12.08	7.25	8.53	7.35	*9.46*
	240 kg N/ha	11.99	10.08	11.87	7.13	8.71	6.88	*9.44*
	Mean	***10.39***	***9.27***	***11.16***	***6.16***	***7.40***	***6.17***	***8.43***
Without ploughing	Control	6.87	7.55	7.64	4.37	3.66	4.75	*5.81*
	120 kg N/ha	11.33	10.12	11.41	6.93	6.82	8.28	*9.15*
	240 kg N/ha	11.82	10.06	11.11	6.96	7.47	7.90	*9.22*
	Mean	***10.01***	***9.24***	***10.05***	***6.09***	***5.98***	***6.98***	***8.06***
$LSD_{5\%}$	Tillage	0.14	ns	0.24	0.14	0.10	0.08	0.60
	Fertilization	0.11	0.09	0.18	0.11	0.09	0.08	0.12
	T × F	0.19	ns	0.32	0.19	0.16	0.13	0.20

ns = non-significant

The yield-increasing effect of fertilization, averaged over the six years examined, differed in the three soil cultivation variants. From the complex variance table (the joint evaluation of the factors) the relationship between fertilization and soil cultivation is shown in Table 2. Linear and quadratic terms were significant for the fertilizer effect, so the relationship can be described well using an optimum curve. The breakdown of the interaction between fertilization and tillage using orthogonal polynomes indicates that in the linear phase, representing initial low fertilizer rates, there is no substantial difference between the three types of soil cultivation. The interaction between the quadratic effect of fertilization and soil cultivation was significant. In optimum fertilizer treatments, however, the differences in yield increase were considerable for all three soil cultivation treatments.

Table 2
Results of variance analysis. Debrecen, 1989–1994

Source of variance	SQ	FG	MQ	F	α-error
Quadratic effect of fertilization	2346.29458	1	2346.29458	2198.22	0.0000
Fertilizer × soil cultivation	15.93738	2	7.96869	7.47	0.0007
m(2)o	217.94437	1	217.94437	204.19	0.0000
m(2)h	7.62189	2	3.81094	3.57	0.0293
m(2)to	18.12171	2	9.06086	8.49	0.0003
m(2)th	2.64059	4	0.66015	0.62	0.6497
m(2)oh	6.75439	2	3.37720	3.16	0.0436
m(2)toh	3.13672	4	0.78418	0.73	0.5689
Deviation	326.61219	306	1.06736		

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CONTRIBUTION OF PLANT COMPONENTS TO SILAGE AND GRAIN PRODUCTION IN BRAZILIAN MAIZE CULTIVARS

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Forage and silage traits were studied using 14 Brazilian maize cultivars chosen on the basis of the harvest index. The contribution of plant components (stalks, leaves, husks, cobs and kernels) to the grain/forage relationship was also evaluated. The major plant components of the forage at the silage stage were on average 37.5% kernels, 21.0% stalks and 18.1% leaves. Husks (14.9%) and cobs (8.5%) were the least expressive. The components with the greatest content of crude protein were the grains (9.65%) and leaves (8.89%), while the other components were less than 3% each. All the maize cultivars evaluated showed good quality characteristics for silage production, with silage pH between 3.71 and 3.98. The proportion of stalk, leaf and husk components was negatively correlated with the cob (r = –0.80, –0.30 and –0.70) and grain (r = –0.76, –0.56 and –0.70) components in the forage dry matter (DM). There was a positive correlation between forage and grain DM yields (r = 0.58). The protein yield was positively correlated with grain (r = 0.98) and forage (r = 0.59) DM yield, and negatively correlated with ear number per plant (r = –0.58). The small variation by genotypes in the protein content, and the absence of any relationship between quality traits and plant components demonstrate the need for an evaluation of genotypes with greater variability for protein content and quality. The differences observed among the cultivars for various plant components demonstrate that forage composition varies as a function of genotype. The value of the harvest index in the evaluation of cultivars needs more information related to digestibility and voluntary intake, while among cultivars of similar productivity, preference should be given to those with higher harvest indexes.

Key words: *Zea mays* L., maize, selection, forage, silage, plant components, harvest index

Introduction

A large amount of research on maize genotypes is done annually in many areas of the world. The principal aim is to evaluate characteristics of agronomic interest for later recommendation to farmers or for utilization in breeding programmes. Nevertheless, the strongest emphasis is given to plants with greater grain yield, resulting in cultivars progressively adapted to this objective. There is a general consensus that genotypes with superior grain yield are also adapted for silage production. According to Hunter and Bizard (1988), this is due to the fact that the grain fraction has higher quality relative to the rest of the plant. Breeders also agree that the selection of hybrids for silage based on grain yield would eliminate the time and costs of separate programmes for breeding and evaluation. It has thus been suggested by Cherney and Cherney (1994) that future improvements in maize silage for animal production will be due less to plant breeding than to refinements in management techniques.

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The success of a plant breeding programme is closely related, among other factors, to the nature of the trait evaluated and above all to the precise identification of the objectives to be achieved. Pollmer (1980) emphasizes that the selection of maize for grain yield does not take into consideration all the genetic potential available from the use of the entire plant when the objective is silage production. In fact important components routinely evaluated to select adapted genotypes for silage production should include the total production of dry matter (DM) of the entire plant per unit area, and of the ears, leaves and stalks. Important relations that should be considered are the ear/leaf and ear/stalk ratios, and the percentage of DM of the entire plant and of individual plant parts. Besides these traits, which are considered to be quantitative in nature, other important aspects with relation to the animals should be evaluated, such as digestibility factors, voluntary intake, and the nutritional quality of the plant parts, thus maximizing the use of a genotype not only for productivity but also for the quality of silage produced.

Studies carried out by various researchers (Vattikonda and Hunter, 1983; Hunter and Bizard, 1988; Gurrath et al., 1989; Barrière et al., 1993) confirm this hypothesis and show that even though there is a significant correlation between grain and forage yield, this relation is not complete. Genetic progress is often reduced due to the negative correlations between favourable morphological characteristics and other factors, such as higher susceptibility to lodging (Le Buanec, 1994). According to Argillier and Barrière (1996), even though differences in the nutrition value of maize forage can be expressed by differences in animal performance (potential for milk production or weight gain), experiments with animals cannot be done routinely in improvement programmes. More refined techniques such as Near Infrared Reflectance Spectroscopy (Le Buanec, 1994) and molecular markers (Morrow et al., 1997) could be used during selection, but often demand greater technology not always available to the plant breeder.

A better understanding of the contribution of nutritional and productive traits and their correlations from the point of view of plant components could be helpful in the selection of maize genotypes to be used in the production of grain, silage, or for both purposes. The objectives of the present research were to study the productivity and quality characteristics of 14 maize cultivars, selected on the basis of harvest index, and to determine the respective contributions of the plant components (stalk, leaves, husks, cob and grains) in the grain/forage relation.

Materials and methods

The trials were carried out at the Centro de Pesquisa de Pecuária do Sudeste (CPPSE), a unit of the Empresa Brasileira de Pesquisa Agropecuária (EMBRAPA), located in the county of São Carlos (central region of São Paulo State) at 21°57' S and 47°50' W. The mean elevation is 856 m, the thirty-year mean annual precipitation is 1476 mm and the mean annual temperature is 19.8°C. The ecosystem is grassland and forest with a tropical montane climate. The experiment was set up on 18 December 1987 on an Oxisol (Udox suborder in the USDA classification; Xantic Ferralsol in the FAO classification).

Soil acidity was corrected by applying 2 t/ha of dolomitic lime and the soil was prepared by one ploughing and two levellings. Fertilization at planting was carried out with 30–60–30 kg/ha of total N, P_2O_5 and K_2O, respectively. Cover fertilization consisted of 40 kg/ha of total N. Other cultivation operations were carried out only when necessary. The statistical design involved randomized blocks with four replications. The plots consisted of seven rows each 5 m long with 1 m spacing. After germination the plants were thinned to a density of 50,000 plants/ha. A useful area of 10 m^2 (2 rows) was used for grain production and 10 m^2 (2 rows) was used for the production of forage, with one row left as a border in the centre and at the edges of the plots.

Fourteen maize genotypes (Table 1) were evaluated chosen on the basis of two years of preliminary results for harvest index (grain DM yield/total DM yield). Four of the genotypes (P-3210, BR-300, P-3216 and G-5555) had indices considered to be high (greater than 0.50), two (DINA 50 and Contimax 133) were medium to high (0.40 to 0.49), three (Phoenyx 2324, Contimax 233 and Phoenyx C) had a medium index (0.30 to 0.39), and three (FO-01, Maya XXIV and FO-Capineira) had a low index (less than 0.29). Cultivars BR-201 and CO-14 were sown for comparative purposes.

Table 1
Cultivars evaluated and the firms producing or supplying them

Cultivars	Source	Supplying firm
BR-201	Double-cross hybrid	EMBRAPA
BR-300	Double-cross hybrid	EMBRAPA
CO-14	Three-way-cross hybrid	Colorado
Contimax 133	Double-cross hybrid	Contibrasil
Contimax 233	Double-cross hybrid	Contibrasil
DINA 50	Double-cross hybrid	Dinamilho
FO-Capineira	Top-cross	Colorado
FO-01	Three-way-cross hybrid	Colorado
G-5555	Double-cross hybrid	Germinal
Maya XXIV	Variety	IAC
Phoenyx 2324	(IAC-1 XXIII x IAC Maya XXIV)	IAC
Phoenyx C	(IAC Maya Itel. x IAC-1)	IAC
P-3210	Double-cross hybrid	Pioneer
P-3216	Double-cross hybrid	Pioneer

The mid-silking date (MS), plant height (PHG) and ear height (EH), lodging (LOD), percentage of broken plants (BP), number of ears per plant (NEP) and percentage of infected ears (IE) were estimated. Plots destined for forage were cut at the silage stage, when the number of days to harvest for forage (FH) and the forage green weight (FGW) were recorded. From a sample of 13 plants in each plot the green and dry weight (48 h in a forced circulation oven at 70°C) of the stalks, leaves, husks, cobs and kernels were measured for ten plants, thus obtaining the proportion of each component in relation to the green and dry weight of the entire plant.

An approximately 1 kg sub-sample from the three remaining plants was chopped and used to prepare sealed plastic silo sacks. All the air was removed with a suction pump and the weight of each sack was noted. Using another sub-sample from these three chopped plants and the components obtained from the other ten plants, the percentage of crude protein was determined by the micro-Kjeldahl method (AOAC, 1970) and calculated on the basis of the percentage of dry matter at 105°C for 12 hours. This analysis permitted the determination of the total percentage of protein in each plant component and in the forage. Six months after being sealed, the sacks were opened to determine final weight, protein levels and pH. The quantity of non-visible loss in the silage was determined as the percentage difference between sample weight prior to and after silaging.

The field parameters evaluated for the forage rows were also recorded for the rows destined for grain production. After harvest the total number of ears, percentage of infected ears, kernel humidity, weight of ears (without husks), kernel weight, and percentage of protein in the kernels was verified. The statistical analyses and the Spearman correlations were done with the SAS program (SAS Institute Inc., 1989).

Results and discussion

The cultivar FO-Capineira was the latest, with 80 days to flowering, and the genotypes P-3210 and BR-300 were the earliest, with 57 days (Table 2). According to Van Waes et al. (1994) the use of very early cultivars with elevated production of dry matter (DM) is a very positive aspect in regions with an unfavourable climate or for the plantation of another crop in quick succession. In Brazil, this aspect has become important in autumn–winter or off-season maize ("safrinha") with the objective of producing maize for grain and/or forage. This type of cultivation is done in a risky environment and demands adapted and early cultivars (Alliprandini et al., 1998).

The cultivars FO-Capineira (264 cm), Phoenyx 2324 (260 cm) and Maya XXIV (245 cm) showed the greatest plant height. FO-Capineira showed the highest ear placement with relation to plant height (0.84), while P-3216 (0.44) and BR-300 (0.45) had the lowest ear placement of all the cultivars. The highest lodging indices were observed in the cultivar FO-Capineira in both the forage plots (2.9%) and the grain plots (19%). In the grain yield plots this fact became irrelevant because this cultivar proved to be unsuitable for this purpose. The cultivar G-5555 showed the highest values for broken plants in the forage plots (8%). In the grain production plots Phoenyx C (26%), Maya XXIV (18.9%) and G-5555 (17.5%) showed the highest indices of broken plants; the mean for all cultivars was 11.5% (Table 2). This also shows the care necessary in the selection of cultivars for silage, based exclusively on the total DM yield, in the case of later double-purpose use. Hunter and Bizard (1988) have emphasized that in spite of the fact that plants destined for silage are removed earlier than those destined for grain production, the chance of damage due to lodging prior to silaging increases at higher planting densities.

The dry matter (DM) trait showed a mean of 44% for forage, 42% for silage and 86% for grain. According to Daynard and Hunter (1975), when the plants reach 35% of whole plant DM this should represent about 85 to 90% of the maximum grain yield. An elevated ear number per plant was obtained for the cultivar FO-Capineira (10.7), which differed statistically from the others, but these ears were smaller, with few kernels per ear and with insignificant cobs. This can be explained by the use of the *Teosinte* genetic background in this three-way-cross hybrid.

The highest value of infected ears (IE) was found in cultivar P-3216, with one-third of its ears affected. The cultivars with lowest IE were CO-14 (3.6%) and Contimax 233 (3.8%), which were statistically different from the others. According to Hunter and Bizard (1988) disease resistance is an important trait which should be considered in silage breeding programmes.

Table2

Traits[1] of 14 maize cultivars evaluated for grain, forage (For) and silage (Sil) production

Cultivars	MS (days)	FH (days)	PHG (cm)	EH/ PHG	LOD (%)		PB (%)		EP	IE (%)	Dry Matter (%)			SNVL (%)	pH
					For	Grain	For	Grain			For	Grain	Sil	Sil	Sil
BR-201	61	103	178	0.50	0.5	7.3	1.0	15.7	1.1	7.5	53	88	51	0.73	3.93
P-3210	57	103	184	0.48	0.0	0.5	0.0	2.9	0.9	14.2	43	86	41	0.56	3.76
DINA 50	67	103	212	0.54	0.9	2.6	1.5	2.9	1.1	16.9	43	86	41	0.56	3.77
Contimax 133	64	105	214	0.52	0.0	2.8	0.5	4.8	1.0	10.3	46	88	46	0.53	3.75
Phoenix 2324	70	118	260	0.64	0.0	4.9	2.0	11.1	1.2	14.6	46	85	43	0.82	3.77
Contimax 233	70	109	226	0.63	1.9	11.0	3.4	7.8	1.3	3.8	42	86	41	0.42	3.74
CO-14	69	109	218	0.62	1.1	5.5	1.0	16.0	1.3	3.6	41	86	38	0.65	3.74
BR-300	57	103	171	0.45	0.5	2.3	1.5	11.9	1.1	6.7	56	89	52	0.93	3.98
FO-01	69	105	230	0.73	0.4	4.1	0.8	16.7	2.4	17.8	42	90	41	0.58	3.85
P-3216	60	105	208	0.44	0.0	5.4	2.0	4.3	1.0	30.9	41	81	41	0.46	3.74
MayaXXIV	70	112	245	0.63	0.5	5.8	1.0	18.9	1.3	16.4	38	83	38	0.30	3.72
G-5555	61	109	210	0.54	2.9	3.7	8.0	17.5	1.2	9.2	45	87	42	1.16	3.78
Phoenix C	64	112	239	0.62	0.9	6.0	1.4	26.0	1.2	9.8	41	86	41	0.29	3.71
FO-Capineira	80	118	264	0.84	2.9	19.0	0.6	4.2	10.7	10.2	36	87	37	0.56	3.76
Mean	66	108	219	0.59	0.9	5.8	1.8	11.5	1.9	12.3	44	86	42	0.61	3.79
C.V.(%)	2	0	7	4.43	47.0*	46.6*	62	28.4	9.3*	20.5*	8	3	8	63.00	1.90
D.M.S.[2]	2	0	20	0.36	2.2	7.1	4.4	8.5	1.3	7.7	5	4	5	0.55	0.10

*Values obtained from original data transformed according to ($\sqrt{x+0.5}$).

[1]FF = Mid-silking; FH = Forage harvest; PHG = Plant height; EH/PHG = Ear height/plant height ratio; LOD = Lodging; BP = Broken plants percentage; EP = Ears per plant index; IE = Infected ears; SNVL = Silage non-visible losses; pH = hydrogenoxide potential.

[2]Minimum significant difference covering two means by the Duncan 5% probability test.

Silage non-visible losses (SNVL) were generally low. G-5555 (1.16%) and BR-300 (0.93%) showed slightly higher loss rates than the other genotypes. The values obtained for silage pH, which averaged 3.79, showed low variability over the cultivars, and these values fell within the standard described by Silveira (1975), who considered a pH lower than 4.2, among other factors, to classify silage as good.

Using a basis of 35% of DM for the forage yield (Table 3), the cultivar Phoenyx 2324 was the most productive (51,032 kg/ha), differing statistically from the others. BR-300 (25,002 kg/ha) and FO-Capineira (31,176 kg/ha) were the least productive. The values of forage and protein yield for these cultivars followed the same order.

Table 3
Yield traits[1] of evaluated cultivars (kg/ha)

Cultivars	Forage plots			Grain plots			HI
	FDMY	CFY35%DM	FPY	GDMY	CG14.5%H	GPY	
BR201	12,741	36,403	807	4733	5535	448	0.38
P3210	11,922	34,064	861	4769	5577	415	0.42
DINA50	12,351	35,290	890	5350	6257	476	0.43
CX133	13,277	37,933	891	5725	6696	550	0.43
Phoenix2324	17,861	51,032	1020	6464	7560	627	0.36
CX233	13,204	37,726	849	5547	6488	550	0.42
CO14	12,189	34,825	804	4478	5238	427	0.37
BR300	8,751	25,002	621	4330	5065	433	0.49
FO-01	11,948	34,137	818	4225	4942	398	0.36
P3216	12,083	34,524	825	5271	6165	459	0.43
MayaXXIV	13,624	38,927	823	5402	6318	532	0.39
G5555	13,088	37,396	905	5543	6483	544	0.43
Phoenyx C	13,693	39,124	885	6353	7430	628	0.46
FO-Capineira	10,912	31,176	697	1499	1753	138	0.15
Mean	12,689	36,254	835	4978	5822	473	0.40
C.V. (%)	18	18	19	16	16	18	17
D.M.S.[2]	3219	9198	228	1167	1365	125	0.09

[1]FDMY = Forage dry matter yield; CFY35%DM = Corrected forage yield at 35% of DM; FPY = Forage protein yield; GDMY = Grain dry matter yield; CG14.5%H = Corrected grain yield at 14.5% of humidity; GPY = Grain protein yield; HI = Harvest index.
[2]Minimum significant difference covering two means by the Duncan 5% probability test.

The cultivars with the highest grain yields (at 14.5% grain moisture content) were Phoenyx 2324 (7,560 kg/ha) and Phoenyx C (7,430 kg/ha). FO-Capineira was the least productive (1,753 kg/ha). A similar order was observed for grain DM yield (GDMY) and grain protein yield (GPY) for these cultivars. The low grain yield of FO-Capineira resulted in the lowest harvest index (HI = 0.16), which differed significantly from the others. The cultivars with the highest indices were BR-300 (0.49) and Phoenyx C (0.46). Nevertheless, the highest indices found here were slightly below the mean of those shown by Dhillon et al. (1990).

In comparison with the pre-established levels (Material and methods) no cultivar showed a high harvest index. Those with indices previously considered high showed a medium to high HI in this research. The cultivars FO-01 and Maya XXIV previously mentioned as having low HI showed medium values in these trials. These results are in accordance with Dhillon et al. (1990), who demonstrated significant genotype-environment interactions for DM yields in silage and grain harvests.

The analysis of the contribution of different plant parts collected at the silage stage (Table 4) showed that on average the kernels (37.5%) had the highest participation in plant dry matter weight, followed by the stalks (21%) and leaves (18.1%). Husks (14.9%) and cobs (8.5%) were the components that had the lowest contribution. According to these data the grain to stover ratio can be estimated as 0.60 (considering the mean of all cultivars). This was below the range described by Hunter and Bizard (1988), who stated, however, that this ratio might be lower in very short-season areas, as it was in the present case due to late planting.

Table 4

Contribution rate of plant components (stalks, leaves, husks, cobs and kernels) in forage cultivars evaluated for forage at the silage stage (%)

Cultivars	Stalks	Leaves	Ears		
			Husks	Cobs	Kernels
BR-201	15.7	21.0	13.2	9.7	40.4
P-3210	17.2	21.6	13.4	9.8	38.1
DINA 50	20.8	20.9	13.2	10.5	34.5
Contimax 133	20.9	17.8	15.8	8.7	36.8
Phoenix 2324	23.2	16.7	13.2	8.4	38.5
Contimax 233	21.8	14.9	17.6	8.0	37.6
CO-14	20.5	13.7	19.1	8.0	38.7
BR-300	14.4	19.6	12.2	10.4	43.3
FO-01	23.5	18.8	18.8	7.4	31.5
P-3216	19.0	16.6	10.1	10.7	43.6
Maya XXIV	22.0	20.4	13.1	9.1	35.4
G-5555	20.0	11.0	12.7	9.9	46.5
Phoenyx C	21.2	17.6	12.4	8.3	40.5
FO-Capineira	33.1	23.5	23.5	0.1	20.0
Mean	21.0	18.1	14.9	8.5	37.5
C.V. (%)	11.1	9.7	17.5	7.9	5.4
D.M.S.[1]	3.3	2.5	3.7	1.0	2.9

[1]Minimum significant difference covering two means by the Duncan 5% probability test.

For BR-201, P-3210, DINA 50 and BR-300 the contribution of the leaves was greater than that of the stalks. FO-Capineira had the highest percentages of stalk (33.0%), leaves (23.5%) and husks (23.5%), but very low indices for kernels (20%) and cobs (0.1%). These low values are explained bz the similarities with the Theosinte ears. The genotypes with the highest percentage of kernels were G-

5555 (46.5%) and P-3216 (43.6%), while the greatest volume of cobs was produced by P-3216 (10.7%), DINA 50 (10.5%) and BR-300 (10.4%). A greater proportion of leaves than stalk was also observed by Batista et al. (1997) for BR-201 and FO-01 in October sowing, while BR-201 was superior in kernel percentage and forage digestibility to FO-01 and BR-106.

The differences observed between the cultivars with respect to the content of the various plant components show that forage composition may vary as a function of genotype. According to Van Waes et al. (1994), higher ear content in the silage leads to better digestibility. The decrease in this content in relation to total DM in new silage varieties could make it difficult to produce high-quality silage cultivars in the future.

The highest levels of crude protein in the forage DM (Table 5) were obtained with cultivars P-3210 (7.26%) and DINA 50 (7.21%). With relation to the silage, P-3210 (7.76%) and BR-300 (7.65%) had the highest protein levels. Cultivar Phoenyx 2324 showed the lowest crude forage protein content (5.73%), but also showed the highest protein yield due to its high DM yield (Table 3).

Table 5

Percentage of crude protein in forage, silage and plant components (stalks, leaves, husks, cobs and kernels) of 14 maize cultivars

Cultivars	Forage	Silage	Stalk	Leaves	Ears		
					Husks	Cobs	Kernels
BR201	6.36	6.73	2.42	9.04	2.46	2.50	9.57
P3210	7.26	7.76	3.07	10.09	2.64	2.59	9.87
DINA50	7.21	7.32	2.93	9.60	2.99	2.59	8.81
CX133	6.68	6.35	2.81	8.32	3.15	2.36	10.11
Phoenix2324	5.73	6.35	2.51	7.63	2.45	2.40	9.55
CX233	6.43	6.84	2.54	10.05	2.52	2.32	10.29
CO14	6.58	7.51	2.64	9.82	3.12	2.38	10.06
BR300	7.12	7.65	3.25	8.33	2.42	2.32	9.76
FO-01	6.85	7.11	2.98	7.77	3.47	2.52	9.58
P3216	6.83	6.84	3.11	9.26	2.59	2.39	8.50
MayaXXIV	6.03	6.43	2.81	9.27	2.50	2.51	9.61
G5555	6.96	6.90	3.29	10.81	2.51	2.02	10.11
Phoenyx C	6.43	7.25	2.73	7.51	3.17	2.06	9.89
FO-Capineira	6.43	6.88	2.14	7.03	4.85	0.00	9.40
Mean	6.64	7.00	2.80	8.89	2.92	2.21	9.65
C.V. (%)	7.44	7.77	17.06	13.62	21.99	11.26	6.57
D.M.S.[1]	0.70	0.78	0.68	1.73	0.92	0.36	0.91

[1]Minimum significant difference covering two means by the Duncan 5% probability test.

The range observed for the percentage of protein in the kernels was not large, though the cultivars showed statistically significant differences. The cultivars with the highest values had between 10.29 and 9.76% crude protein in the kernels (Contimax 233, G-5555, Contimax 133, CO-14, Phoenyx C, P-3210 and BR-300), while the cultivar with the lowest value was P-3216, with 8.5%. Kaan et al. (1980), in an evaluation of 175 European maize populations, found a protein percentage varying between 8.9 and 14.7%.

The components with the highest values of crude protein were the kernels (9.65%) and the leaves (8.89%), with the exception of cultivars P-3210, DINA 50, P-3216 and G-5555, where higher protein levels were found in the leaves. Other plant parts gave relatively similar results, with low levels (less than 3% for the majority of cultivars). Pollmer (1980) emphasized the importance of evaluating kernel and leaf components as well as the correlations between them, when the objective is the improvement of maize for silage production.

The correlations shown in Table 6 indicate that in general the late cultivars, which have longer periods to mid-silking (MS) and forage harvest (FH), had higher percentages of stalks (r = 0.81 and 0.68) and husks (r = 0.67 and 0.32), but lower percentages of cobs (r = –0.77 and –0.64) and kernels (r = –0.79 and –0.40). The ratio of ear insertion in the stalk (EH/PHG) was negatively correlated with grain DM yield (GDMY; r = –0.33) and with the percentage of kernels (r = –0.76) and cobs (r = –0.83) in the plant and positively with the percentage of stalks (r = 0.82) and husks (r = 0.65). This indicates that genotypes where the principal ear is inserted lower down on the stalk showed a tendency for greater grain yield with a lower proportion of stalk and husks in the plant. The percentage of husks was negatively correlated (r = –0.27) with the number of infected ears (IE). This correlation, although the values are not high, shows the importance of selecting cultivars with good ear covering by the husks for silage production. No important correlation was found between the lodging indices (LOD and BP) and the plant components, or with grain, forage or protein DM yields (GDMY, FDMY or GPY, FPY).

The DM proportion of the stalk, leaves and husks components was negatively correlated with the cob (r = –0.80, –0.30 and –0.70) and grain (r = –0.76, –0.56 and –0.70) components in the plants. These relations are important for the selection of genotypes because they influence the quantity of lignin and the digestibility of the silage produced. Considerable variability was found between the hybrids for these components by Vattikonda and Hunter (1983). These results also support the need for separate evaluation trials for maize grown for whole plant silage production as opposed to grain production, as suggested by Hunter and Bizard (1988).

There was a positive linear correlation (r = 0.58) between forage and grain DM yields (FDMY and GDMY). Thus, on average, cultivars that showed a high forage yield also showed a high grain yield, but though this correlation has been regularly described in the literature, this does not mean that genotypes with elevated grain yield are always the most adapted for silage production (Pollmer, 1980; Vattikonda and Hunter, 1983; Gurrath et al., 1989; Geiger et al., 1992).

Significant positive correlations were found between the harvest index (HI) and the GDMY (r = 0.68), the percentage of cobs (r = 0.67) and the percentage of kernels in the plant (r = 0.64). Thus, the lack of correlation with FDMY indicates the validity of the harvest index for the evaluation of cultivars in forage production. Further study will be required to evaluate digestibility and intake, although among cultivars that possess the same DM yield, preference should be given to those with the highest HI. Weak or even absent correlations between HI and forage yield were also reported by Gallais et al. (1976) and Fairey (1980).

Table 6

Phenotypic correlations[1] between evaluated traits[2] for the forage, silage and grain production of 14 maize cultivars

	GDMY	FDMY	%Stalks	%Leaves	%Husks	%Cobs	%Kernels	GPY	FPY
MS	–0.41	0.10	0.81	0.14	0.67	–0.77	–0.79	–0.35	–0.06
FH	–0.09	0.35	0.68	–0.07	0.32	–0.64	–0.40	–0.02	0.12
PHG	0.14	0.52	0.75	–0.01	0.25	–0.59	–0.46	0.18	0.36
EH/PHG	–0.33	0.15	0.82	0.15	0.65	–0.83	–0.76	–0.26	0.02
LOD (forage)	–0.17	0.02	0.23	–0.11	0.14	–0.25	–0.10	–0.14	0.03
BP (forage)	0.22	0.17	0.02	–0.34	–0.19	0.09	0.26	0.25	0.16
LOD (grain)	–0.32	–0.10	0.34	0.02	0.43	–0.52	–0.32	–0.26	–0.14
BP (grain)	0.12	0.03	–0.12	–0.32	–0.09	0.11	0.29	0.17	–0.01
IE	0.02	0.08	0.05	0.18	–0.27	0.16	–0.03	–0.04	0.13
NEP	–0.62	–0.14	0.73	0.39	0.61	–0.91	–0.77	–0.58	–0.18
GDMY	–	0.58	–0.29	–0.35	–0.56	0.53	0.55	0.98	0.57
FDMY	–	–	0.29	–0.12	–0.34	0.04	0.07	0.59	0.90
% Stalks	–	–	–	0.14	0.44	–0.80	–0.76	–0.23	0.22
% Leaves	–	–	–	–	0.03	–0.30	–0.56	–0.38	–0.15
% Husks	–	–	–	–	–	–0.70	–0.70	–0.52	–0.38
% Cobs	–	–	–	–	–	–	0.79	0.46	0.14
% Kernels	–	–	–	–	–	–	–	0.54	0.12
FDMPT	–0.12	–0.40	–0.22	–0.05	–0.07	0.24	0.14	–0.15	0.04
GDMPT	0.27	0.24	0.13	–0.31	–0.10	–0.06	0.17	0.45	0.17
SDMPT	–0.12	–0.29	–0.14	0.02	0.04	0.13	0.01	–0.13	–0.10
HI	0.68	–0.15	–0.66	–0.28	–0.44	0.67	0.64	0.65	–0.09
Ph	–0.16	–0.17	–0.31	0.24	–0.14	0.12	0.13	–0.14	–0.22

[1]Values greater than or equal to 0.270 were considered significant at the 5% probability level.

[2]MS = Mid-silking (days), FH = Forage harvest (days), PHG = Plant height (cm), EH/PHG = Ratio of ear height to plant height, LOD = Lodging (%), BP = Broken plants (%), IE = Infected ears (%), NEP = Number of ears per plant, GDMY = Grain dry matter yield (kg/ha), FDMY = Forage dry matter yield (kg/ha), FDMPT = Forage dry matter protein (%), GDMPT = Grain dry matter protein (%), SDMPT = Silage dry matter protein (%), HI = Harvest index, GPY = Grain protein yield (kg/ha), FPY = Forage protein yield (kg/ha).

The grain protein yield (GPY) was negatively correlated with the number of ears per plant (r = –0.58), with MS (r = –0.35), and with the percentage of leaves (r = –0.38) and husks (r = –0.53). This indicates that an increase in the number of ears per plant could cause a reduction in the quantity of protein produced by the kernels per unit area. These results confirm the claims of Hunter and Bizard (1988), who found that the ideal maize hybrid for silage should possess a single ear on a single stalk. The tendency of late and fibrous cultivars to show a lower protein yield in the kernels was also verified by Maggiore et al. (1980).

The values of forage, grain and silage protein content and pH indicate that there is little correlation between these traits and plant components. This fact suggests the need for more study with genotypes that have greater variability with relation to level and quality of available protein. Grain protein yield (GPY) was positively correlated with GDMY (r = 0.98), FDMY (r = 0.59), HI (r = 0.65) and the percentage of kernels in the plant (0.54). The forage protein yield (FPY) was positively correlated with GDMY (r = 0.57) and with FDMY (r = 0.90). These data indicate that the production of protein per area was directly related to grain and forage dry matter production.

Conclusions

The differences observed between the cultivars for various plant components demonstrate that forage composition varies as a function of genotype. There was a negative correlation between the contribution of the stalks, leaves and husks and those of the cob and kernel components in the plant dry matter. These results also support the need for separate evaluation trials for maize grown for whole plant silage production as opposed to grain production, especially in areas where silage is the most important objective.

A positive, significant correlation was observed between forage and grain DM yield, while the small variability of the evaluated cultivars with respect to protein levels and the absence of correlation between quality factors and plant components indicated the need for the evaluation of genotypes with more variation in protein level and quality.

Protein yield was closely related to grain and forage DM yield, while the cultivars that had a greater number of ears were later and had higher leaf percentages with a lower grain protein yield. Cultivars where the principal ear was inserted lower down on the stalk showed a tendency for greater grain yield.

The validity of the harvest index for the evaluation of cultivars in forage production requires further study, to including work on voluntary intake and digestibility. In the case of cultivars that have the same forage dry matter productivity preference should be given to those with the highest harvest indices.

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EFFECTS OF DIFFERENT TECHNOLOGICAL FACTORS ON THE INTENSITY OF GROWTH IN HEIFERS

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The growth of Holstein-Friesian heifers up to 6 months of age was examined. The calves were placed individually in barns or outdoor pens and weaned 50 days after birth. The food consumption and body weight of the calves were measured in winter and summer. The body weight gain of the calves did not decrease but increased after weaning from milk replacer. The growth rate decreased more in summer than in winter. The weight gain and growth rate were most intensive between 2–4 months of age. This period was the most favourable for the compensatory growth of the calves.

Key words: growth, development, food consumption, weight gain, barn, pens, calves, heifers

Introduction

It is a frequently experienced fact that heifers kept indoors or outdoors in individual pens during the period of milk or milk replacer feeding exhibit a considerable break in their later development. This temporary "break" may last for as much as one or two months. This "milk-meat" loss is unacceptable nowadays as it prevents the slow but continuous development of the heifers. Therefore it is necessary to elaborate a suitable technology and to determine the optimal growth intensity from the breeding point of view.

The principle factor affecting the growth of the calves is nutrition. In this respect, it is very important to obtain valuable data on the outdoor system widespread in Hungary. Meinershagen et al. (1981) and Blackmer (1982) reported good results in calf rearing in individual pens. One feed per day is able to satisfy the nutritional needs of calves in this form of housing (Szűcs et al., 1975).

It is unclear, however, how these methods influence the further body weight gain and growth intensity of the calves mainly through food consumption.

The aim of the present examinations was to establish the growth intensity of calves feeding once a day and provided with a limited amount of milk replacer when kept in outdoor or indoor individual pens until weaning at 50 days of age.

Outdoor housing is excellent as a temporary replacement for barns and may also lead to an interruption in the infection chain. Outdoor rearing improves the resistance of the calves and decreases mortality (Ballasch et al., 1981), and is excellent for the prevention of complex calf diseases (Ballasch et al., 1983).

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The purpose of calf rearing is not absolutely to get maximum growth (Czakó et al., 1977). Compensatory growth has more practical importance. As is well known, tissues and organs which have not yet reached the state of maximum growth may be able to compensate for the drawbacks of limited nutrition in the next period of life. However, calves which do not have sufficient food-consuming capacity cannot adequately compensate for the effects of limited nutrition suffered at 3–4 months of age (Everitt et al., 1977; Dennan et al., 1979; O'Donovan, 1984). The other important reason why they cannot compensate for earlier drawbacks is the lack of fat deposition. Particularly up to 3–4 months of age, disorders of the internal organs could become permanent. Löhnert et al. (1987) found that calves were often unable to compensate for weight loss caused by disease before 3 months of age. Traumas occurring during the period of the intensive development of internal organs may result in reduced body size unless the food consumption capacity of the calves is improved. Otherwise, the increasing ability for body weight gain after weaning (Bailey, 1986) cannot be utilised.

Skeletal development becomes intensive from 4 months of age. Deficiencies in skeletal growth, irreversible bone deformations, and changes in bone size are quite frequent at this age (Kanagawa et al., 1986; Coleman and Evans, 1986). These disorders may be aggravated by nutritional errors during heifer rearing. Petrescu et al. (1972) found that calves compensated for the consequences of a single daily milk feed by 5–6 months of age.

Precise data on body weight gain, which has age-related possibilities and limits, are essential for the utilisation of growth capacity. The possibilities and limits must be discovered if the development processes of heifers are to be understood. It is known that surplus nutrition provided at young age later results in fat deposition in the mammary tissues (Roy, 1983). Surplus energy consumption during puberty, when the allometric growth of the udder takes place, may decrease milk production later (Little et al., 1979). Serjsen et al. (1982), however, were unable to demonstrate this harmful effect after puberty. Sandles et al. (1987) stimulated the development of mammary tissue by hormone treatment at 2.5 months of age, but this had no effect on the subsequent milk production. According to Brelin et al. (1985) the period from 3–6 months of age is critical for udder development. On the basis of data recorded by Tucker (1987), the allometric development of mammary tissue is intensive by 4–6 months of age, although this development is not accompanied by considerable growth but may be decreased by nutritional errors. Newbold et al. (1987) also reported on the intensive need for body weight gain from 2 months of age in Holstein-Friesian heifers.

Measurements made by Kertz et al. (1987) suggest that 0.9–1 kg/day body weight gain between 3 and 6 months of age does not lead to fat deposition in the mammary tissue. Serjsen et al. (1982), however, found that 1200 g daily body weight gain could cause irreversible damage in mammary tissue.

The relationship between age and body weight becomes stable only in 4-year-old cattle (Marrow et al., 1978). This relationship is not as close in calves,

so different technologies may produce different weights at the same age. These weight differences will generally be equalised after 4–5 months of age. The development of calves is also influenced by housing and climatic factors. Therefore, limited nutritional and housing conditions were applied which had a direct impact on the intensity of development. The aim of the experiments was to obtain valuable data on the effects of technological factors on calf development. Studies were made on the extent to which adequate body weight gain and continuous development could be ensured under sub-optimum conditions.

Materials and methods

The experiments were carried out on a herd of 650 Holstein-Friesian cows. Twenty female calves from the same progeny group of a sire were placed in individual outdoor pens (group 1) with a surface area of 3 m^2/animal and twenty calves were kept in individual indoor pens (group 2, control) with the same surface area/animal. Rearing took place at the same time and according to the same methods, with one daily feed between 10 and 50 days of age. Subsequently, after a week's training, rearing was continued in groups, indoors, under identical circumstances, with 3 m2/animal surface area. The experiments were carried out in a single replication during two phases, in June–August and January–March. The Mancini test (Mancini et al., 1965) was used to control the colostrum supply of the calves. The antibody and the IgG antigen were made by Phylaxia. Blood samples were taken 1, 7, 13, 25 and 49 hours after birth and subsequently on the 9th, 14th, 21st and 50th days.

The food consumption and body weight gain of the calves were measured simultaneously. The growth rate of the calves was recorded up to 6 months of age. The air temperature and relative humidity in the barns were recorded continuously during the whole period of the experiment. The air temperature fluctuated between –14.5 and +8.5 °C in winter, and between +13.5 and +34 °C in summer. Notes in the tables, such as "summer", "winter", "outdoors" and "indoors" refer to the season and circumstances of initial rearing.

After analysing the data of this experiment, 6 groups were set up to study the development of the calves. The groups differed from each other in the housing (outdoors-indoors), the season (summer–winter) and in the frequency of feeding (once or twice daily). The calves were weighed daily and the mean body weights over 10-day periods were used later in the calculations.

The growth rate was calculated according to the method of Kovács et al. (1983). After the logarithmic transformation of the data, the rate of growth was calculated for each period of development. Linear regression and the fitting of the transformed data to the regression line were calculated. Coefficient "k", describing the steepness of the lines, is in direct proportion to the growth rate and has the same mean as coefficient "b" in the case of allometric growth. The rate of body weight gain differs according to the species, breed and technological method, but usually exceeds the allometric growth rate of the body frame.

If this coefficient exceeds 1, it indicates rapid development. The limits of the growth phases are determined by the dispersion zone of the data at the fracture points of the transformed lines. Data outside this zone fitted well to the calculated lines. Therefore, data showing the limits of a phase indicate a change in the body weight rather than determining it exactly.

Results and conclusion

There was a significant difference in the food consumption between the groups of calves up to 50 days of age. Animals in the group kept outdoors consumed more fodder ($P<0.05$) and more hay ($P<0.01$) (Tables 1 and 2).

Table 1
Food consumption of calves until the age of 50 days in summer

Studied features	Unit	Group 1, housed outdoors				Group 2, housed indoors			
Age in days	day	10-20	20-30	30-50	10-50	10-20	20-30	30-50	10-50
Milk replacer, sum total	kg	6.00	6.00	6.55	18.55	6.00	6.00	6.50	18.50
	s.d.	0.47	0.71	1.51	0.89	0.68	0.80	1.21	0.89
Daily consumption	kg	0.60	0.60	0.32	0.46	0.60	0.60	0.32	0.46
Concentrate, sum total	kg	1.55	1.75	7.51	10.81	1.27	1.24	6.35	8.86
	s.d.	0.45	1.14	2.09	2.36	0.36	0.73	1.50	2.13
Daily consumption	kg	0.15	0.17	0.37	0.27	0.12	0.12	0.31	0.22
Alfalfa hay, sum total	kg	1.58	4.63	13.65	19.86	0.99	1.68	5.87	8.54
	s.d.	0.31	1.82	2.54	2.36	0.47	0.61	3.19	3.71
Daily consumption	kg	0.15	0.46	0.68	0.49	0.09	0.16	0.29	0.21

Table 2
Food consumption of calves until the age of 50 days in winter

Studied features	Unit	Group 1, housed outdoors				Group 2, housed indoors			
Age in days	day	10-20	20-30	30-50	10-50	10-20	20-30	30-50	10-50
Milk replacer, sum total	kg	6.00	6.00	6.50	18.50	6.00	6.00	6.50	18.50
	s.d.	0.41	0.81	0.93	0.71	0.79	0.93	1.02	0.91
Daily consumption	kg	0.60	0.60	0.32	0.46	0.60	0.60	0.32	0.46
Concentrate, sum total	kg	1.66	1.29	6.41	9.36	2.05	3.56	10.93	16.99
	s.d.	0.57	0.53	1.40	1.90	0.57	1.22	3.33	4.42
Daily consumption	kg	0.16	0.12	0.32	0.23	0.20	0.35	0.54	0.42
Alfalfa hay, sum total	kg	1.81	4.93	14.85	21.59	1.35	1.45	8.15	10.95
	s.d.	0.76	1.62	4.79	6.80	0.37	0.96	3.41	4.42
Daily consumption	kg	0.18	0.49	0.74	0.45	0.13	0.14	0.40	0.27

Table 2 shows that calves housed indoors in barns (Group 2) consumed less hay and fodder than their herdmates housed outdoors in winter. This difference was significant at the $P<0.01$ level. The hay consumption of calves housed outdoors between 30 and 50 days of age considerably exceeded that of the group kept in the barn. The reason for this could be the more active behaviour of calves kept outdoors, which spent more time moving and consuming food. It is well known that young calves eat roughage (hay) by preference, earlier than grain fodder under optimal circumstances. This tendency can be seen in the hay consumption data of Group 1. The increase in hay consumption was also stimulated by mutual learning, since the calves could see each other outdoors while this was limited in the barn.

A study of the seasonal effect shows that there was no significant difference between the winter and summer food consumption of calves kept outdoors (Group 1), while the winter grain fodder consumption of calves kept indoors was significantly higher than in summer and their hay consumption increased slightly in winter. This tendency was sometimes more pronounced, in agreement with the work of Schingoethe et al. (1986), who reported an increase in dry matter consumption in cold weather. The food consumption of calves housed differently in the milk feeding period did not differ greatly later when they were kept in groups (Table 3).

Table 3
Average food consumption of calves between 60 and 180 days of age

	Unit	Group 1			Group 2		
Age	day	60	120	180	60	120	180
Consumption							
In summer							
Fodder	kg	1.50	2.00	2.50	0.83	3.33	2.78
Maize silage	kg	–	1.50	2.00	0.44	1.67	2.22
Alfalfa silage	kg	2.00	3.80	3.60	1.67	3.33	3.33
In winter							
Fodder	kg	1.40	2.00	2.50	1.50	2.50	3.50
Maize silage	kg	–	1.00	2.00	0.40	1.00	1.50
Alfalfa silage	kg	2.64	2.00	2.95	1.50	3.00	3.50

Since the calves consumed different amounts of food at the beginning, there was a pronounced difference between the amounts of nutrition consumed by the two groups, as illustrated in Table 4. The amount of nutrients consumed by the two groups kept outdoors generally exceeded that of groups kept in barns. In summer the group kept in the barn (Group 2) achieved better results, while in winter the performance of the outdoors group was somewhat better, though the difference was not significant.

Table 4
Nutrient consumption between 10 and 50 days of age

	Unit	In summer		In winter	
		outdoor group	indoor group	outdoor group	indoor group
Dry matter					
daily consumption	kg	0.99	0.89	1.00	0.84
sum total	kg	40.89	36.53	41.16	34.74
Digestible crude protein					
daily consumption	kg	0.24	0.15	0.24	0.19
sum total	kg	9.87	6.45	9.99	8.18
Crude fat					
daily consumption	kg	0.09	0.08	0.09	0.09
sum total	kg	3.83	3.47	3.85	3.69
ME*	MJ	13.6	10.56	14.03	13.43
NEM** daily consumption	MJ	5.40	3.38	5.17	5.21
NEG*** daily consumption	MJ	2.12	2.46	2.29	2.22
Protein concentration	%	22.10	22.45	22.22	21.56

*ME = metabolisable energy; **NEM = net energy maintenance; ***NEG = net energy gain

The body weight gain of calves kept outdoors was higher in winter than in summer. This tendency was not observed in animals housed in barns. The reason for this could be that cattle adapt to a low temperature more easily than to a higher one (Worthington, 1977). Ficher et al. (1985) did not find this correlation in their experiment, but Broucek et al. (1986) noted that calves reared outdoors consumed slightly more nutrient in winter.

Table 5 shows that the frequency of diarrhoea and pneumonia was less in the case of outdoor housing and the symptoms were milder and visible for a shorter time than in animals kept in the barn. The colostral supply is essential for subsequent periods of life in calves. This colostral supply was sufficient particularly in calves born in winter.

Table 5
Colostral supply, frequency of diseases and mortality in calves

Studied features	In summer		In winter	
	Group 1 (outdoors)	Group 2 (indoors)	Group 1 (outdoors)	Group 2 (indoors)
IgG level				
above 15 mg/ml	18	11	16	18
5–15 mg/ml	3	5	2	2
below 5 mg/ml	–	4	2	–
0 mg/ml	–	1	–	–
Diarrhoea cases at the age of				
6–8 days	–	9	3	–
10–35 days	12	11	2	14
50 days	12	20	6	13
Pneumonia up to the 50th day of age	2	3	–	2
Immunity at 6–10 days of age (IBR, Adeno, VD, PI-3)	+	+	+	+
Mortality up to the 50th day of age	–	–	–	–
after 50 days of age	–	2	–	–

There was no significant difference during development between the body weights of calves housed outdoors or in barns. According to the data shown in Tables 6a and 6b the body weights of 60-day-old calves kept in barns were slightly higher than those of their herdmates reared outdoors ($P<0.01$).

In respect to the body weights of 60-day-old calves, winter results were generally better than those measured in summer. Hot weather in the summer, especially, decreased the body weight of 60-day-old calves, while cold weather tended to lead to better performance.

Table 6a
Body weight gain of calves until 60 days of age (group kept outdoors)

Studied features	Unit	Age (day)					
		3–10	10–20	20–30	30–40	40–60	3–60
In summer							
Average weight gain	kg	1.49	3.39	3.97	5.01	6.71	20.88
Coefficient of variation		97.90	54.20	50.10	44.70	38.50	21.70
Average daily gain	g	214	339	397	417	372	348
Body weight at 60 days of age	kg	–	–	–	–	–	64.28
In winter							
Average weight gain	kg	1.68	4.88	7.03	7.00	9.59	30.57
Coefficient of variation		64.80	36.40	31.50	32.50	25.90	20.70
Average daily gain	g	241	488	703	583	532	509
Body weight at 60 days of age	kg	–	–	–	–	–	69.72

The initial backwardness in the body weight of the calves becomes obvious when compared to that of other animals. According to the US Standard, to be classified as "good" the body weight should be 73–90 kg at 2 months of age and 181–203 kg at 6 months of age in Holstein-Friesian calves. The average body weights of these two age groups were 83.9 kg and 171 kg in a survey carried out by Heinrichs et al. (1987).

Table 6b
Body weight gain of calves until 60 days of age (group kept indoors)

Studied features	Unit	Age (day)					
		3–10	10–20	20–30	30–40	40–60	3–60
In summer							
Average weight gain	kg	1.60	2.86	4.21	4.12	6.11	18.2
Coefficient of variation		94.30	55.50	48.60	49.20	43.60	23.50
Average daily gain	g	229	286	421	343	339	300
Body weight at 60 days of age	kg	–	–	–	–	–	63.92
In winter							
Average weight gain	kg	1.30	4.84	5.28	5.24	10.55	26.60
Coefficient of variation		97.60	35.30	38.00	39.30	20.00	30.80
Average daily gain	g	187	484	528	436	586	444
Body weight at 60 days of age	kg	–	–	–	–	–	73.20

It can be concluded that the housing method had no great influence on weight gain. This conclusion is supported by Ficher et al. (1985) and Runev et al. (1984). Body weights measured at 2 months of age in summer were 20 kg less than the required value, which may be due to the high temperature of this season. This represented a considerable growth deficiency which was not compensated for by the calves until 6 months of age. Ideally the calves should reach a body weight of 75–80 kg by the time they are weaned. Body weight

gain, as a general index, conceals an important feature of development, the fact that the different organs and tissues develop at a different pace. For instance, an improvement in the solid food consumption capacity of the calves depends not only on the developmental stage of the rumen and the digestive tract, but also on the functioning of the nervous system, which develops intensively at this age. This is indicated in the present experiment, where the calves began to consume other foods earlier and their solid food consuming ability developed more rapidly when fed with a limited amount of milk replacer.

As the effect of limited feeding with milk replacer, the weight gain of outdoor-housed calves up to an age of 60 days was 350–500 g greater on average than in the case of indoor-housed calves.

The results also indicate the better utilisation of the postweaning growing ability of the calves. As Tables 7a and 7b show, the body weight gains of the calves varied, but were all above 1000–1200 g/day.

Table 7a
Body weight gain of calves until 180 days of age (group kept outdoors)

Studied features	Unit	Age (day)					
		60–90	90–120	120–150	150–180	50–180	3–180
In summer							
Average weight gain	kg	20.25	20.75	32.19	28.85	114.54	128.28
Coefficient of variation		38.70	34.10	27.90	24.80	14.00	11.90
Average daily gain	g	680	860	1070	960	829	712
Body weight at 60 days of age	kg	–	–	–	–	–	172.3
In winter							
Average weight gain	kg	14.76	31.97	37.07	29.35	125.27	144.27
Coefficient of variation		54.20	29.60	29.60	35.60	14.00	11.30
Average daily gain	g	490	1070	1240	980	907	819
Body weight at 60 days of age	kg	–	–	–	–	–	185.9

Table 7b
Body weight gain of calves until 180 days of age (group kept indoors)

Studied features	Unit	Age (day)					
		60–90	90–120	120–150	150–180	50–180	3–180
In summer							
Average weight gain	kg	18.93	25.94	32.64	32.93	116.55	129.34
Coefficient of variation		29.30	24.10	19.70	20.40	13.80	14.20
Average daily gain	g	630	860	1090	1100	867	738
Body weight at 60 days of age	kg	–	–	–	–	–	179.92
In winter							
Average weight gain	kg	27.36	28.65	34.28	37.86	124.66	152.77
Coefficient of variation		22.40	17.40	12.40	13.70	11.70	9.80
Average daily gain	g	910	960	1140	1260	1006	848
Body weight at 60 days of age	kg	–	–	–	–	–	203.12

The more uniform rate of body weight gain in the group housed outdoors in winter indicates that an increased rate of body weight gain in 4-month-old cattle can be achieved earlier by improving the rearing conditions.

It was found in earlier experiments (Guszter et al., 1985) that the intensive postweaning growth rate decreased if the adaptation to solid food had not occurred and if cold milk had been fed, or if the milk replacer diets exceeded 8–10% of the body weight. In the present experiment there was no substantial drop in the postweaning growth due to the satisfactory solid food consumption ability of the calves. The body weight data indicating the limits of each growth phase are shown in Table 8.

Table 8

Limits of growth phases with calves raised in different technologies during the milk replacer period

Groups	Unit	Phase 1		Phase 2	
		min.	max.	min.	max.
One daily feed with milk replacer					
Outdoors					
in summer	kg	61.66	63.10	120.23	125.89
	s.d.	6.58	5.49	13.91	12.87
in winter	kg	60.26	63.10	123.03	131.83
	s.d.	7.11	6.91	14.65	15.77
Indoors					
in summer	kg	60.26	61.66	128.82	134.90
	s.d.	6.40	5.86	15.49	16.01
in winter	kg	64.57	67.61	117.49	125.89
	s.d.	7.47	6.91	12.98	14.43
Mean	kg	61.69	63.87	122.39	129.63
Two daily feeds with milk replacer					
Outdoors					
in summer	kg	61.66	63.10	107.15	112.20
	s.d.	6.32	5.46	14.08	13.96
in winter	kg	62.65	66.07	109.03	114.04
	s.d.	7.08	6.11	15.61	14.31
Mean	kg	62.15	64.58	108.09	113.12
Summed means	kg	61.84	64.11	117.63	124.13

Table 9 contains the regression equations of the growth phases. The whole period can be characterised by coefficients of growth greater than 1 because of the high growth rate up to 6 months of age.

Between phases (generally at 50–60 days of age) there is a considerable change in rate. The second most intensive growth phase was found to be between 110 and 130 days of age. Following this phase the intensity of growth somewhat decreased.

Table 9
Growth rate at different ages

Groups	Phase of growth	Coefficient of growth: 'k-value'	Regression equation	Correlation coefficient
Outdoors	I	1.0769	y=1.6078+0.0322x	0.997 ***
in summer #	II	1.0937	y=1.5724+0.0389x	0.995 ***
	III	1.0690	y=1.7100+0.0290x	0.994 **
Outdoors	I	1.0914	y=1.5840+0.0380x	0.978 **
in winter #	II	1.1033	y=1.5452+0.0427x	0.998 ***
	III	1.0641	y=1.7740 +0.0270x	0.992 **
In the barn	I	1.0592	y=1.6590 +0.0250x	0.988 **
in summer #	II	1.0984	y=1.5486 +0.0408x	0.999 ***
	III	1.0715	y=1.7100 +0.0300x	0.999 ***
In the barn	I	1.0819	y=1.6469 +0.0334x	0.995 ***
in winter #	II	1.1040	y=1.6090 +0.0430x	0.997 ***
	III	1.0801	y=1.7075 +0.0335x	0.993 **
Outdoors	I	1.1641	y=1.5200 +0.0660x	0.996 ***
in winter ##	II	1.0806	y=1.6736 +0.0337x	0.997 ***
	III	1.0616	y=1.7782 +0.0260x	0.995 **
Outdoors	I	1.1066	y=1.5850 +0.0440x	0.955 *
in summer ##	II	1.0934	y=1.5856 +0.0388x	0.994 **
	III	1.0772	y=1.6629 +0.0323x	0.994 **

#: one daily feed with milk replacer; ##: two daily feeds with milk replacer

The intensity of growth was influenced by the nutrition intake of the calves and by weaning from milk replacers. Therefore the calculated limits of the growth phases indicate only approximately that the weight gain changed its intensity according to the early level of nutrition.

The reason for this early intensive growth was probably the artificially limited weight gain at the beginning. This is proved by the greater growth coefficients in this stage, which decreased only slightly in the next phases of growth, indicating great growing ability. The results suggest that great growing ability is present from 2 months of age in the Holstein-Friesian breed.

The results of Heinrichs et al. (1987), based on a huge database, point to the possibility of utilising the postweaning growing phase at 2–4 months of age. According to their data, the body weights of Holstein-Friesian heifers in the US have improved mainly between 1 and 3 months of age over the past 30 years. This was attributed partly to the increase in body weight at birth and partly to better early nutrition.

The present experiments support the hypothesis that body weight has an important role, even greater than that of age, in the development of heifers. The required weight is strictly controlled when the animal is involved in breeding. However, it must be emphasised that a knowledge of the preceding growth rate is indispensable for judging the importance of weight at a given age. The growth rate of heifers can be well controlled by weighing at birth, and at 2, 4 and 6 months of age. It is recommended that the conditions of the animal should be evaluated at the same time as weighing. It can be concluded on the basis of the results that an intensive increase in body weight can be stimulated at 2–4 months of age. The relatively high growth intensity biologically possible at this age can only be utilised if the calves possess the necessary dry matter consuming ability.

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Short communication

GENETICS OF EARLY FLOWERING MUTANTS IN TRITICALE

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By applying gamma rays and EMS, individually and in combination, early flowering mutants were isolated in the M_2 generation. The mean reduction in days to flowering and days to maturity in the mutants over the control was about 12–18 days and 11-18 days, respectively. F_1 hybrids were similar to the parents in days to flowering. F_2 plants segregated in a 15:1 ratio for normal to early flowering. The F_3 plants gave a segregation of 8:7 for segregating to non-segregating families. A test cross showed a 3:1 ratio for normal to early type. The results suggest that the early flowering mutant in triticale is controlled by two recessive, non-allelic genes.

Key words: early flowering mutants, triticale

Introduction

Improvement in either a single or a few polygenically controlled economic traits and quality attributes is not normally achieved by hybridization with the least deleterious effect within a short time. Alternatively, targeted recombination can be achieved within a short time without very much disturbing the yield and quality status of existing, well-adapted varieties by induced mutations (Reddy et al., 1994). The present paper reports the induction of mutants with early flowering and a study of their genetic behaviour in the hexaploid triticale Borba.

Materials and methods

Seed materials of the hexaploid triticale Borba were treated with gamma rays (10 kR, 20 kR, 30 kR), EMS (0.5%) (6h, 8h, 10h) and a combination of gamma rays and EMS (10 kR+10h; 20 kR+8h; 30 kR+6h). Individual spikes of each M_1 plant were sown one spike to a row in the M_2 generation. Mutatnts showing early flowering/maturity were isolated in segregating M_2 rows. The mutants were reciprocally crossed with the parent variety Borba. The F_1, F_2 and F_3 generations from these crosses were raised. A test cross was also conducted to confirm the results. Data on days to flowering and days to maturity were recorded in the parents and in different generations. The chi-squared test was conducted to test the goodness of fit for the segregation pattern.

Results and discussion

A total of 9 early flowering/maturity mutants were isolated in M_2 spike progenies. The mean number of days to flowering and maturity was significantly reduced in the mutants. The mean reduction in days to flowering and days to

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Table 1

Frequency distribution of days to flowering in parents, F_1 and F_2 generations of the cross between the hexaploid triticale variety Borba and its early flowering mutant (EFM)

Population	No. of days to flowering					Total plants	Mean days to flowering	Observed frequency		Probability (P)
	–30	–35	–40	–45	–55			Control	Early	(15:1)
Borba	–	–	–	36	41	77	49.5	–	–	–
EFM	8	32	1	–	–	41	33.6	–	–	–
Borba × EFM										
F_1	–	–	2	18	14	34	48.6	34	–	–
F_2	16	53	4	146	891	1110	–	1041	69	0.95–0.98

Table 2

Frequency distribution of days to maturity in parents, F_1 and F_2 generations of the cross between the hexaploid triticale variety Borba and its early flowering mutant (EFM)

Population	No. of days to maturity								Total plants	Mean days to maturity	Observed frequency		Probability (P)
	75–80	–85	–90	–95	–100	–105	–110	–115			Control	Early	(15:1)
Borba	–	–	–	–	4	41	29	3	77	107.6	–	–	–
EFM	3	37	1	–	–	–	–	–	41	84.5	–	–	–
Borba × EFM				–						–			
F_1	–	–	–	–	–	21	13	–	34	106.9	34	–	–
F_2	2	63	2	–	8	41	982	12	1110	–	1043	67	0.70–0.80

maturity in the mutants over the control was about 12–18 days and 11–18 days, respectively. No significant differences were noticed in other agronomic characters between the mutant and the control. Results pertaining to the genetics of the early flowering mutant in the triticale variety Borba are presented in Tables 1-3. All the 34 F_1 hybrids obtained were similar to the parent Borba in days to flowering and days to maturity. Of the 1110 F_2 plants, 69 plants flowered earlier, giving a ratio of 15 normal to 1 early flowering/maturing variant. Of the 135 randomly selected F_2 plants with normal flowering, 72 families showed segregation, giving a ratio of 8 segregating to 7 non-segregating. Among the 47 test cross progenies that were produced only eleven exhibited mutant (early) character.

Table 3

Segregation for days to flowering and days to maturity in F_3 families and testcross progeny of a cross between the hexaploid triticale variety Borba and its early flowering mutant

Progeny			Ratio/Probability	Mutant type
F_3	72 (segregating)	63 (non-segregating)	8:7 (1.00)	Digenic recessive
Testcross	36 (normal control type)	11 (early flowering)	3:1 (0.80-0.90)	Digenic recessive

Normal flowering in F_1 hybrids resulting from reciprocal crosses between the parent triticale variety Borba and its early flowering mutant suggests the recessive nature of the mutant character. The segregation of the F_2 plants in a 15:1 ratio for normal flowering to early flowering suggests the epistatic interaction of two non-allelic genes, where mutant character is controlled by two genes, both of which should be present in the recessive condition if the mutant character is to be expressed. The F_2 ratio of 8:7 for the segregating to non-segregating families and the test cross ratio of 3:1 for normal to mutant confirms that the mutant character is inherited as a digenic recessive and that both the normal genes are epistatic to recessive mutant non-alleles. Similar results in the reciprocal crosses also suggest the absence of any cytoplasmic effects.

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Short communication

EFFECT OF TIME OF SUBMERGENCE AND NUTRIENT MANAGEMENT PRACTICES ON DIRECT-SOWN RICE IN A SEMI-DRY ECOSYSTEM

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A field experiment was conducted from 1993 to 1995 to study the influence of the time of submergence and management practices on direct-sown semi-dry rice. Maximum productivity can be accomplished by applying seed treatment and foliar spray with 1% KCl along with a basal application of 25% N, superphosphate-enriched farmyard manure and potash, in the event of delayed release of canal water even up to 45 DAS for direct-sown semi-dry rice.

Key words: semi-dry rice, submergence, nutrient, growth/yield attributes

Introduction

Rice is a semi-aquatic plant with a high degree of adaptability to changing conditions. The system of rice cultivation in various rice-growing regions of the country therefore varies largely depending upon the soil and climatic conditions prevalent in the region. The principal systems of rice culture are dry, semi-dry and wet.

Semi-dry rice culture is a system associated with upland and lowland rice in the early and late stages, respectively. The major barriers to improving the productivity of rice in this system are moisture stress at different stages, greater percolation loss and inefficient use of plant nutrients. The drought tolerance of many plants could be increased by subjecting them to moderate dehydration for several days. In this context, an experiment was conducted to study the influence of nutrient management practices at different times of submergence on semi-dry rice.

Materials and methods

Field experiments were carried out at Tamil Nadu Rice Research Institute, Aduthurai during the wet season (August – January) of 1993–94 and 1994–95 to study the influence of nutrient management practices at different times of submergence for maximizing the productivity of direct-sown semi-dry rice var. Cr 1009. The experiments were laid out in a split plot design in three replications. The time of submergence [I_1 – Submergence 30 DAS (days after sowing); I_2 – submergence 45 DAS and I_3 – submergence 60 DAS] was assigned to the main plots. Nutrient management practices [S_1 – Seed treatment and foliar spray with 1% KCl along with superphosphate-enriched farmyard manure (FYM) and potash; S_2 – seed treatment and foliar spray

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with 1% KCl along with basal application of 25% N, superphosphate-enriched FYM and potash; S_3 - basal application of superphosphate-enriched FYM and potash; S_4 – basal application of 25% N, superphosphate-enriched FYM and potash; S_5 – absolute control] were assigned to the subplots. The soils of the experimental sites are given in Table 1. The soils are moderately drained deep clay, taxonomically called odorthenric chromusterts alluvial clay. They are low to medium in available N, medium in available P and K with a neutral reaction. A seed rate of 100 kg ha^{-1} was adopted. The seeds were treated with one per cent potassium chloride (KCl) for 16 hours, then air dried in the shade to the original seed moisture content (12%) and stored. Seeds treated with KCl were sown in the plots in line, adopting an interrow spacing of 20 cm, anticipating the monsoon rains. Superphosphate at a rate of 60 kg P_2O_5 ha^{-1} was enriched with 750 kg ha^{-1} farmyard manure (FYM). Muriate of potash at a rate of 60 kg ha^{-1} was applied basally prior to sowing. Nitrogen was used at a rate of 150 kg ha^{-1}, 25 per cent of which was applied basally as gypsum neem-coated urea. (Prilled urea can be mixed with gypsum and neem in the ration of 5:4:1 and kept for 24 h prior to application. The remaining N doses were applied in the form of prilled urea as top dressing in 4 splits on an equal nitrogen basis after submergence. A foliar spray with 1.0% KCl was given 30 DAS. A surfactant, Tee Pol B 300, was added at a rate of 1.0 ml l^{-1} spray solution.

Table 1
Soil characteristics of the experimental field

Characteristic	1993–94	1994–95
Organic carbon (%)	0.46	0.38
Available N	204.00	220.00
Available P_2O_5	14.20	13.80
Available K_2O	248.50	256.20

Results and discussion

Seed treatment with 1% KCl, with the basal application of 25% N, superphosphate-enriched FYM and potash (S_2), registered the highest root length, root:shoot ratio and vigour index of the seedlings (Table 2). This might be due to the effect of changes caused by the seed treatment in the physico-chemical properties of the cytoplasm, and to the greater hydration of the colloids, the higher viscosity and elasticity of the protoplasm, the greater amount of bound water and the more intense metabolism. The consequences of these cellular changes are claimed to induce a greater xeromorphic structure, more intense transpiration, lower water deficit, the ability to retain a greater quantity of water and a more efficient root system. This is in agreement with the findings of Genkel and Badanova (1959).

Early submergence was favourable for the leaf area and thereby a higher leaf area index was obtained. The reduced leaf production rate, leaf expansion, cell size and intercellular volume under a limited moisture supply restricted the size of the leaf area. O'Toole and Baldia (1982) reported that leaf area expansion was more sensitive to moisture stress. Dry matter accumulation also responded to the submergence regimes. The greater accumulation of dry matter in the case of early flooding was due to the favourable growth components, such as LAI (leaf area index). A larger number of panicles and filled grains was also recorded under early flooding. The better crop growth achieved under early flooding appears to

have utilised all the available resources and increased the yield attributes and grain yield. There was a 13.0% reduction in grain yield under late submergence (60 DAS) compared to early submergence. Supplying adequate N, superphosphate-enriched FYM and potash may have increased the foraging capacity of the plants in search of moisture and nutrients, resulting in the production of more and larger leaves, thus contributing to higher dry matter production (DMP) (Table 3).

Table 2
Seedling characters (15 DAS) as influenced by the treatments

Treatment	Root length (cm)	Root:shoot ratio	Seedling vigour index	Root length (cm)	Root:shoot ratio	Seedling vigour index
	1993–94			1994–95		
			Main plot (Submergence)			
S_1	4.21	0.311	1769	4.38	0.337	1826
S_2	4.28	0.313	1763	4.39	0.342	1814
S_3	4.25	0.313	1788	4.28	0.311	1835
SED	0.05	0.006	22	0.05	0.009	32
CD	NS	NS	NS	NS	NS	NS
			Subplot (Nutrient management)			
S_1	4.87	0.338	1890	4.89	0.350	1943
S_2	5.28	0.443	2075	5.46	0.442	2120
S_3	3.51	0.239	1721	3.65	0.296	1771
S_4	4.31	0.337	1892	4.51	0.346	1959
S_5	3.25	0.206	1287	3.23	0.216	1331
SED	0.07	0.005	20	0.06	0.014	23
CD	0.13	0.011	40	0.12	0.028	46

Table 3
Growth characters as influenced by the treatments

Treatment	LAI at flowering	DMP at flowering*	Relative leaf water content**	LAI at flowering	DMP at flowering*	Relative leaf water content**
	1993–94			1994–95		
			Main plot (Submergence)			
S_1	4.81	8678	86.28	5.14	7835	85.66
S_2	4.75	7948	69.08	5.15	7843	76.41
S_3	4.14	7385	68.26	4.61	7445	75.60
SED	0.08	59	2.26	0.11	58	0.39
CD	0.22	163	6.28	0.30	161	1.09
			Subplot (Nutrient management)			
S_1	4.76	8395	79.40	5.19	8409	81.52
S_2	5.09	8784	79.55	5.56	8793	82.39
S_3	4.76	8351	71.92	5.10	8322	78.24
S_4	4.96	8551	71.95	5.30	8572	78.81
S_5	3.28	4937	69.87	3.67	4440	75.16
SED	0.03	66	0.61	0.07	62	0.29
CD	0.06	131	1.21	0.13	123	0.58

*kg/ha; ** %

Early N application was inferior to late application, particularly when most of the nitrogen was applied at sowing. Apparently, the roots of the plants are not well developed in the early stage of crop growth. In order to promote the quicker growth of vigorous roots, the basal application of N as a starter dose is felt essential. This is also reflected in the seedling quality characters when 25% N is applied as a basal dose.

P and K are not considered necessary for the promotion of vegetative growth, but it is possible that these nutrients had an indirect influence on the uptake of N, which in turn might have increased the growth attributes. This is in confirmation with the findings of Venkatasubbiah et al. (1982). Under dry conditions, N use efficiency was greatly improved by the application of gypsum-coated urea (Panigrahi and Dixit, 1991).

The availability of P in upland soil is lower than in flooded soils (Patrick and Mahapatra, 1968). Hence, P deficiency may be the limiting factor under upland conditions. The basal application of compost treated with superphosphate gave a higher uptake of P, as reported by Mahapatra and Srivastava (1984).

A larger number of panicles and filled grains was also observed after the combined application of basal N as gypsum neem-coated urea with superphosphate-enriched FYM and potash, plus seed and foliar treatment with KCl (Table 4). This is in conformity with the findings of Mishra and Pyare Lal (1994). Similarly, increased grain yield was noticed with the above treatment. The possible causal factor could be the quicker and normal germination of rice seeds with increased seedling vigour, resulting in the better utilisation of fertilizer N, P and K.

Table 4
Yield attributes and grain yield as influenced by the treatments

Treatment	Panicle No. m^{-2}	No. of filled grains	Grain yield kg ha^{-1}	Panicle No. m^{-2}	No. of filled grains	Grain yield kg ha^{-1}
	1993–94			1994–95		
			Main plot (Submergence)			
S_1	284.4	84.2	4718	303.0	83.0	4894
S_2	297.2	82.9	4590	300.4	82.1	4788
S_3	270.8	74.9	4101	277.8	73.2	4255
SED	4.8	1.0	75	3.2	0.9	103
CD	13.5	3.0	209	9.1	2.6	286
			Subplot (Nutrient management)			
S_1	303.3	85.2	4954	316.9	83.2	5126
S_2	350.7	92.4	5347	351.4	90.0	5513
S_3	300.6	85.0	4902	313.5	83.4	5096
S_4	330.6	88.0	5143	331.4	86.3	5297
S_5	149.4	52.1	2004	155.5	54.3	2197
SED	3.5	1.0	77	3.5	0.8	73
CD	7.0	2.1	154	7.0	1.7	146

The application of KCl spray 30 DAS improved the water status of the plants by increasing the relative leaf water content, and subsequent flooding 45 DAS led to increased growth and yield attributes. Thus, in the case of early flooding at 30 and 45 DAS, the basal application of N, superphosphate-enriched FYM and potash along with seed and foliar treatment with KCl showed a significant increase in growth and yield parameters. Delayed submergence beyond 60 DAS resulted in water stress at critical growth stages, which was ultimately reflected in a lower grain yield. This was also supported by the finding of Cruz et al. (1986) that poorer crop growth due to delayed flooding could not be recouped even after the stress was relieved.

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Review

REFORM ENDEAVOURS IN THE 1960S AND 1970S*

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The period 1966–1975 was the most successful in the post-war history of Hungary's agriculture. The agricultural policy-makers developed step by step an agricultural model specially designed for Hungary. In agriculture the year 1967 played a decisive role in the reform process. In that year a new minister, Imre Dimény, was appointed to head the Ministry of Agriculture and Food; cooperative land ownership was established, agricultural cooperatives and state farms were authorised to carry out non-agricultural activities, and market-oriented, private, small-scale production was recognised by policy-makers. The agricultural leadership took steps to break the state monopoly of external trade. In the 1970s technically operated production systems spread in Hungarian agriculture. It is worth mentioning that in 1973 the first textbook on the economics of food production appeared. The period 1966–1975 shows very clearly the great importance of personalities. This period also holds some lessons for the present. One of these is that the agricultural policy-makers of the time wanted not simply to copy foreign models, but to improve them.

Key words: agribusiness, cooperative ownership, non-agricultural activities, great personalities.

Introduction

There are several reasons for my looking back to the agricultural policy of the 1960s and 1970s. One is the presence in our midst of the 75-year-old Imre Dimény, who was Minister for Food Economy from 1967 to 1975 and had a decisive role, with Professor Ernõ Csizmadia, in the development of the Hungarian agricultural model. The other is the present dismal state of Hungarian agriculture, which lends topicality to this retrospect, since the period 1966–75 holds many lessons for the present.

When dealing with reform trends, only the most important reform steps will be mentioned, as time will not permit a complex treatment.

Reform steps and endeavours in the 1960s

Professor **John Davis** (1956) wrote a study in Boston (USA) using input-output analysis to demonstrate backward and forward linkages in American agriculture. Among other things he came to the following conclusion:

It is characteristic that whereas 30 years ago American agriculture produced 70–80% of its production tools itself, nowadays it buys at least 50%

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of them on the market. According to Western literature, today agriculture is so much interconnected with industrial, trading, transportation and storage activities that we cannot speak about agriculture in the old sense, but have to use the term agribusiness. This branch of economy, "agribusiness", employs 40% of the total labour force and produces 40% of the Gross National Product in the United States.

This new approach increasingly spread in Hungary in the early sixties and in 1967 it resulted in several reform steps.

In the spring of this year, after some preliminary work, the Ministry of Agriculture and Food was created as the result of an amalgamation between the Ministry of Agriculture, the Ministry of Food, the National Forestry Board and the National Office of Land and Cartography. The former workforce was reduced by 50%. The ministry was headed by Imre Dimény. His main endeavour was to co-ordinate and review the whole food chain from the industrial background to the consumer. The structure and operational guidelines of the new ministry were elaborated under his guidance. Within a short time he succeeded in giving meaning to the framework of the ministry and started to solve the existing problems at great speed

In this same year, 1967, Hungary became a member of the FAO and a Centre for State Farms was set up on entrepreneurial principles.

In May 1967 a conference of Secretaries of State met to discuss preparations for the National Agricultural Fair and Exhibition. One basic principle was that, in line with the momentous changes in the regulatory system of Hungarian food production, the vertical integration of food production (production of raw material, processing, consumption and export) should be demonstrated in its entirety. This should be the chief message of the main pavilion.

The most important event of 1967 was undeniably the September session of Parliament. At this session Minister Imre Dimény, at the request of the Council of Ministers, introduced Bills about cooperatives and about land property and use. It should not be forgotten that this session was held a few months before the implementation of the New Economic Mechanism in 1968. In his exposée the Minister argued for the passing of the two laws. His most important argument was that cooperatives are large farms functioning as an industrial enterprise. They can charge the value of circulating capital, the amortization of fixed assets and the rent of their lands to the costs of production. Their business autonomy reinforces their entrepreneurial character. It is therefore desirable to enlarge the proportion of cooperative property. This aim was served by the Bill on land property and use. If passed it would allow the cooperatives to acquire, in a differentiated manner and in return for reimbursement, ownership of the lands in their use. The essence of the Bill was to gradually unify cooperative land property and land use. The other Bill was aimed at the reinforcement of the auxiliary activities of cooperatives in

order to improve the utilisation of their resources and income-earning capacity. Care was taken to reassure political leaders and representatives of the industrial lobby that large farms were not attempting to replace large-scale state industry. This was not the intent of the bill, and would anyway bye an irrealistic goal. Large farms would continue to be engaged primarily in food production. The exposée by the Minister also dealt with the relationship between the cooperative farms and the household plots of the members, and with their organic unity. The institution of the household plot was not a preliminary but a long-term element in the economic policy concept. The bill stated that farms and household plots constituted an organic whole. The Minister claimed that the two bills would contribute to the ability of Hungarian agriculture to utilise the possibilities offered by the new regulatory system.

As mentioned above, in 1967 the Centre of State Farms (CSF) was set up on entrepreneurial principles. The task of this centre was to regulate the state farms while respecting their autonomy, i.e. to replace the previous direct regulation by indirect regulation. It is characteristic of the working style and speed of the new Minister and Ministry that by December 1967 the first lessons to be drawn from the functioning of the CSF, the reorganizations and the new regulations were already being evaluated. Three elements of this evaluation should be stressed:

– "Direct interference in the activities of state farms should be avoided..."
– "Mergers between farms are not intended; at the present stage of the reform state farms should not be burdened with this..."
– "The main task in the present period is for the state farms to become acquainted with the economic incentives of the reform and to take advantage of them."

It is remarkable to note that by June 1967, a few months after its inception, the new Ministry has already turned its attention to the export-import activities of firms. It came to the conclusion that the mutual financial incentives of producers and foreign trade firms should be reinforced and that export-import licences should be granted for special products: breeding eggs, grain, stallions, race-horses, flower-seeds, etc. By the end of 1967 four food processing firms had obtained autonomous foreign trade licences for certain products.

The seventies

In the first half of the seventies there were several important reform steps in the area of food economy. Three of these will be singled out here.

The Ministry of Agriculture and Food (MAF) was well aware that if food production was to be successful three main links in the agribusiness chain should be co-ordinated. In the early seventies several negotiations were held between the MAF and the Ministry of Metallurgy and Engineering (MME). In 1973 the two ministries formulated a common suggestion, the starting point of

which was the dispersed nature of the organization, management and regional location of the domestic production of agricultural machinery, often leading to parallel activities. According to data from 1972 firms attached to the MME and the MAF each had a 50% share in the domestic market for agricultural machines. In order to rule out parallelism and to elaborate a unified agricultural machinery development concept it was necessary to change the organization. It was thus proposed that from 1st January 1974 the central regulation of agricultural machinery production should be transferred to the MAF. After this date the MAF would also be responsible for international co-operation in the area of agricultural and food processing machinery. The production of food processing machines would be gradually shifted to the MAF with a deadline of 31st December 1975. Besides these organizational changes the suggestion also provided for the financial preconditions required for the initiation of technological development in the Hungarian production of agricultural machinery. By October 1974 a suggestion had been submitted to the Ministerial Council centred on the modernization of production technologies. To establish the program financially, a two-billion Forint investment fund set up by the firms would be supplemented by a one-billion Forint contribution from the budget and a 2.6 billion Forint long-term loan. This experiment was abandoned after 1st January 1976, so it could not be evaluated. Nevertheless it was of interest in an international context. There is no knowing what would have happened if had been implemented in full.

Another point which should be mentioned is the relationship between foreign trade and the food economy. In the first years of the 1970s there were several rounds of negotiations between the MAF and the Ministry of Foreign Trade (MFT), in the course of which the MAF was asked to extend the range of foreign trade licensing at firm level. These negotiations were basically an effort to break the foreign trade monopoly of the state, but at the time with no success. It was not until 1988 that his dream became reality.

Finally mention should be made of the spread of technically operated production systems /TOPS/ in the Hungarian economy in the first part of the 1970s. The majority of these production systems functioned as true centres of innovation looking for answers to the needs of practice, not as enclaves of Marxist philosophy. They pumped complex technologies into the firm structure of agriculture. In reality they were the main carriers of technological development. With the spread of production systems a considerable growth in Hungarian agriculture was achieved, as will be briefly described below.

Hungarian agriculture and economic growth

It is generally known that economic growth was dynamic in Hungarian agriculture in the 1960–70s but there are considerable differences as to the output indicator used (Table 1).

Table 1
Economic growth in Hungarian agriculture (1960–1989)

Year	Gross[1]	Gross[2]	Net[1]	Net[2]
1960	100.0	100.0	100.0	100.0
1961	98.7	99.6	93.6	94.5
1962	102.3	103.2	99.4	100.3
1963	107.7	109.5	104.6	106.3
1964	112.7	115.2	107.9	110.2
1965	107.4	110.9	97.8	101.2
1966	117.7	122.1	108.1	112.5
1967	122.0	128.2	108.4	114.7
1968	122.8	131.6	108.2	118.1
1969	131.0	143.7	121.6	134.4
1970	124.2	141.4	99.4	116.8
1971	133.4	151.2	105.6	123.7
1972	137.7	156.0	107.3	125.9
1973	147.0	166.2	112.3	132.0
1974	152.9	175.8	111.8	134.5
1975	161.0	185.7	112.1	136.0
1976	157.8	183.4	103.5	127.5
1977	176.4	207.6	115.9	144.1
1978	179.9	215.8	112.1	143.5
1979	178.5	217.9	124.7	138.9
1980	183.4	225.9	111.8	145.2
1981	190.4	241.0	112.6	150.3
1982	199.1	259.9	123.3	170.3
1983	195.9	259.9	118.9	169.5
1984	199.3	264.2	125.1	175.2
1985	189.2	254.8	113.2	163.8
1986	194.6	264.1	119.1	171.5
1987	195.4	275.4	115.5	171.8
1988	201.8	277.7	127.6	188.2
1989	200.5	272.4	131.7	189.5

[1]Considering only plant production + animal husbandry; [2]Including non agricultural activities

If agriculture is compared in a narrow sense (plant production + animal husbandry) or in a broader sense (including the non-agricultural activities of farms) the year 1967 is found to have played a considerable role in both the gross and net output. If non-agricultural activities are taken into account the increase in both indices is greater. The large difference between the growth of gross and net output can be explained partly by efficiency problems. It should also be mentioned that for objective reasons replacement investments had a large share in overall investments.

Within the fifteen years between 1961/65 and 1976/80 the gross agricultural output grew on average by approx. 3% a year. This growth was produced mainly by wheat + maize + pigs + poultry. Another structural indicator of growth is that both large-scale and small-scale agriculture had a part in it.

The Central Statistical Office (1980) and other sources presented the index of gross output in international comparison. In the group of 18 countries Hungary was ranked among the first three on the basis of yearly average rate of growth. As a result of this rapid growth, the grain, meat, etc. production per capita was favourable in an international context and created a balanced domestic food market with enough food for large masses at affordable prices. It was customary for neighbouring countries to look to Hungary if they confronted a problem to see how the Hungarians coped with it. Hungarian agriculture had international renown and contributed to the prestige of the country.

It also pertains to the history of the 1970s that the first textbook on the economics of food production was published (Csizmadia, 1973). The author intended this as a preliminary approach. to be followed by a second, more mature version in the mid-seventies, but this was not to be.

Ernõ Csizmadia was removed from his post in 1974 and spent a year in hospital with a nervous breakdown. Imre Dimény resigned in 1974 and was relieved of his duties in July 1975. He left the ministry building like a leper and was an outcast from society for years. I am proud to say that the Hungarian Academy of Sciences played an active role in the rehabilitation of both.

Finally, it should not be forgotten when evaluating the reform steps and endeavours of the 1970s that it was politics which interfered with the process in the winter of 1972/73 and halted the reform. In the early years of the decade the MAF was under constant criticism, since the income of those living from agriculture surpassed the income of industrial workers. In the mid-1970s small-scale production was discontinued. Were it not for 1967, the agricultural cooperatives might well have been nationalized.

Some thoughts about the role of the human factor

It is difficult to say exactly what part Ernõ Csizmadia and Imre Dimény played in the results attained. To express it in figures is impossible. They can be likened to the "steersmen of the ship". They were true companions in shaping agricultural policy, helping and complementing each other. In their success a role was also played by, crop producers, animal breeders and the agricultural intelligentsia, some of whom are still with us. Thanks are due to them all.

Nevertheless, there are many specific aspects of the activity of Imre Dimény that should be enumerated separately. He was the first minister of food economy in the country and always tried to think in terms of "agribusiness" as a whole. He was responsible for introducing and improving the indirect management system in agriculture, and had a lion's share in the development and reinforcement of the enterprise character of state farms and cooperatives. He laid the foundations for mixed land ownership in Hungarian agriculture, playing an important role in giving cooperative and state property equal rights.

At the September 1967 session of Parliament Ferenc Erdei said that the Bill: "acknowledged ... cooperative property as equal". During his ministership market-oriented, small-scale commodity production gained ground in Hungarian agriculture.

Although a minister is always in part a politician, he was nevertheless held to be a professional, especially as regards technological development. It became a catchword that the era of Dimény was a "golden age" for the mechanisation of Hungarian agriculture.

It is impossible to talk about the well-functioning integration system of Hungarian food production without mentioning Imre Dimény. Large farms and production systems played an important role in this integration system.

He was closely involved in the development of a Hungarian model of cooperatives based on mixed land ownership and mixed activities, pointing in the direction of agribusiness, and made many efforts to break the state monopoly of foreign trade.

During his ministership, parallel with the necessary organisational changes or even before them, he tried to create the financial background necessary for the planned steps. He was in close contact with science, which was not an easy matter, since science offers not only epoch-making suggestions, but mediocre and dilettante ones too. Imre Dimény had a sense for choosing the right suggestion. He made few bad choices. His ministership was characterized by a healthy blend of good and not so good decisions.

The Hungarian agricultural model

To finish with, mention should be made of the important fact that after 1966, as a result of the reformed agricultural policy, a new agricultural model gradually emerged which had several special characteristics.

One is the concept of ownership which was shaped under the direct influence of Ernõ Csizmadia and Imre Dimény. This concept of ownership involved the legal equality of state and cooperative property and the foundation of agriculture on mixed (state, cooperative and private) property relations.

Another strategic characteristic of the Hungarian agricultural model was the recognition of the prospects and importance of small-scale production. At the cost of considerable infighting the right of market-oriented small-scale production to exist in Hungarian society was finally acknowledged. The fact that Ernõ Csizmadia and Imre Dimény fought for this right led to their denigration the mid-seventies.

Another characteristic of the model was the development of non-agricultural activities on farms and the combination of these with normal farm work. As a result of this Hungarian agriculture rested on three pillars (crop production, animal husbandry and non-agricultural activities). The share of non-

agricultural activities within the total production value was the highest in farms working on the worst lands. Today we look back to the debates of those times with a smile.

Finally mention should be made of the outstanding role of production systems in the Hungarian agricultural model. Production systems were on the borderline of scientific and technical progress, attempting to exploit the best results of both and diminishing friction between agriculture and its industrial background.

As a result of the ownership concept in the second half of the 1980s large-scale and small-scale agriculture both had a 50% share in the net value of production.

The fact that a Hungarian model of agriculture exists is little known in the international literature. It is heartening, however, that the knowledgeable agricultural German economist, Professor K. E. Wädekin (1990) acknowledged the existence of such a model. He remarked that Hungarian agricultural policy, although a unique case, may be considered as a new model and appears as such in Soviet and Eastern European publications. Political leaders and specialists from other socialist countries intensively studied this model, especially over the last ten years. "Hungary is representative of a policy which has not overthrown the once-imposed Soviet system, but has introduced so many features of individual or even outright private initiative and of flexibility of management in the social sector ... that her agrarian system may still be called large-scale socialist farming, but certainly not Soviet-type farming." (p. 323).

Science should return to the complex evaluation of the Hungarian agricultural model.

At the end of the 1980s conditions changed considerably. The Hungarian agricultural model should have been developed further, but the new agricultural policy heralded in 1991 chose not the road of organic development but the road of political determination. Hungarian agriculture lost a large part of its production potential and was drowned in chaos.

Lessons for the present

The period discussed here holds many lessons for the present. In an interview given to the daily newspaper "Népszabadság" about the transformation of agriculture (Anonymous, 1989) Imre Dimény said: "First of all the problem should not be approached ahistorically, ignoring recent decades... The starting point should not be a new distribution of land – although those who venture should be given or rented land – but a road of development should be sought starting from the existing situation."

In another interview (Anonymous, 1996) he stated among others things: "A decision is needed as to whether agriculture is a strategic industry in Hungary or not."

He was always consistent in admitting that greater production could only be achieved by a development-friendly agricultural policy.

I think the arguments which motivated the transformation of the Ministry of Agriculture into a Ministry of Agriculture and Food in 1967 are still topical. Without denying that the task of the ministry is different in a market economy than it was after 1967, I still think the experience gained by the MAF should be exploited. A minister can only be successful if he is an agribusiness specialist.

With the recognition and enforcement of the industrial character of state farms and cooperatives the agricultural policy contributed to the efficient use of production factors. The political recognition of market-oriented small-scale production and its economic integration pointed in the same direction. A gradual, evolutionary privatization was achieved without speaking much about it. Instead of national property a combination of state, cooperative and private property was chosen.

The post-1967 agricultural policy developed a three-pillared structure for farms. With a few exceptions, however, foreign trade was absent from the third pillar. In 1988 this too became possible. It would have been a plain agricultural policy step if a large part of the farms had been transformed into agribusiness firms. Thereby Hungary might have taken a large step forward. The agricultural policy of 1991, however, sentenced the cooperative sector and a large part of the state farms to death.

The period investigated has a message for science, too. A textbook should be written on the economics of food production. What is particularly needed is a chapter on the "economics of the food industry". For years Imre Dimény has urged the concentration of large forces on research into the economic problems of the food industry.

Nowadays there is much debate about cooperative land property. Looking at it from the horizon of Hungarian agricultural history, this debate is incomprehensible. September 1967 and the two decades of dynamic growth which followed it have given an answer to this question. What is the sense of so much debating?

An even more momentous question raised by many in the 1960s and again in the 1990s, involves the constraint of profit-oriented management and the cooperative character of cooperative farms, and whether they can be reconciled or not. If the answer is no, the cooperative sector should be left out of the agricultural structure. I think a good starting point might be the debates about the industrial-type management of cooperatives in the 1960s, which led to the conclusion that the cooperative and industrial characters of cooperatives can be reconciled. The industrial management in cooperatives should be reinforced, but at the same time the cooperative character too.

The lesson of the period is that without technological development there is no progress. However, technological development had already halted in the 1980's. The extremes of the new agricultural policy, with the stress laid on full-time family farms, intensified the existing problems, since the capacity utilisation diminished, and the investment needs of the transition rose to peak levels. The capital intensity (capital/production) of family farms based on Western European agriculture is much higher than that of Hungarian agriculture based on the combination of large- and small-scale production. This is a long-term problem.

Finally, the most important lesson for the new generation is not to be half-hearted. Ernő Csizmadia and Imre Dimény did not want to copy. Quite the contrary! There were things they certainly did not want to copy, there were other things they copied. but they did not stop at that. They thought about how to improve things, how to take a step forward. They wanted to outdo the original! Sometimes they succeeded. sometimes not, but what they achieved was at least as good as the original.

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Symposium paper

BIOLOGY OF THE ANGIOSPERM MALE GAMETE AND ITS ROLE IN FERTILIZATION*

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Sperm cells of angiosperms are relatively simple cells that are transported within the pollen to a specific site within the embryo sac. Possessing conditions essential for cellular fusion with the female target cells, the egg and central cells; these sperm cells fuse to produce the zygote and endosperm, respectively. Sperm cells may be identical or may differ in flowering plant species, yet of the two cells, only one of the sperm cells will contribute directly to the genetics of the offspring. Given the observed differences in sperm cells, it is possible that one particular type of sperm cell is much more likely to fuse with the egg cell.

Key words: fertilization, male gamete (angiosperm), *Plumbago*, preferential fusion, tobacco

Successful sexual reproduction in flowering plants requires a carefully orchestrated set of interactions between the male and female gametes within a complex framework of interactions between two gametophytes (gamete-producing tissues) and the enclosing sporophytic tissues of the flower. Interactions between pollen tubes and the floral tissues upon which they elongate are extensively described; the interaction of the gametes is less well understood but equally important to the outcome.

Unlike most male gametes in biology, the sperm cells of flowering plants are incapable of directional movement (Russell, 1991). In fact, they may be capable of slight changes in shape based on microtubule linking, including changes in circumference and shape, which may influence their transport in the pollen tube, but do not constitute movement. The patterns of cross-linking in microtubular arrays do not appear to favour their use in a conventional motility system and in fact may inhibit it. Isolated sperm cells have not been observed to move.

An understanding of how sperm cells are transported within the pollen tube (microgametophyte) has begun to emerge within the last ten years (Russell, 1996). Dynamic parallel cortical arrays of filamentous actin (F-actin) and microtubules are found in sub-distal regions of the pollen tube. These arrays, accompanied by the presence of apparent myosin on the surface of membranes surrounding the vegetative nucleus and generative cell, suggest that actomyosin interactions provide much of the capability of movement for translocation of the male gametes. Experimental evidence using F-actin disruption with cytochalasin B on growing pollen tubes does not completely inhibit translocation, however,

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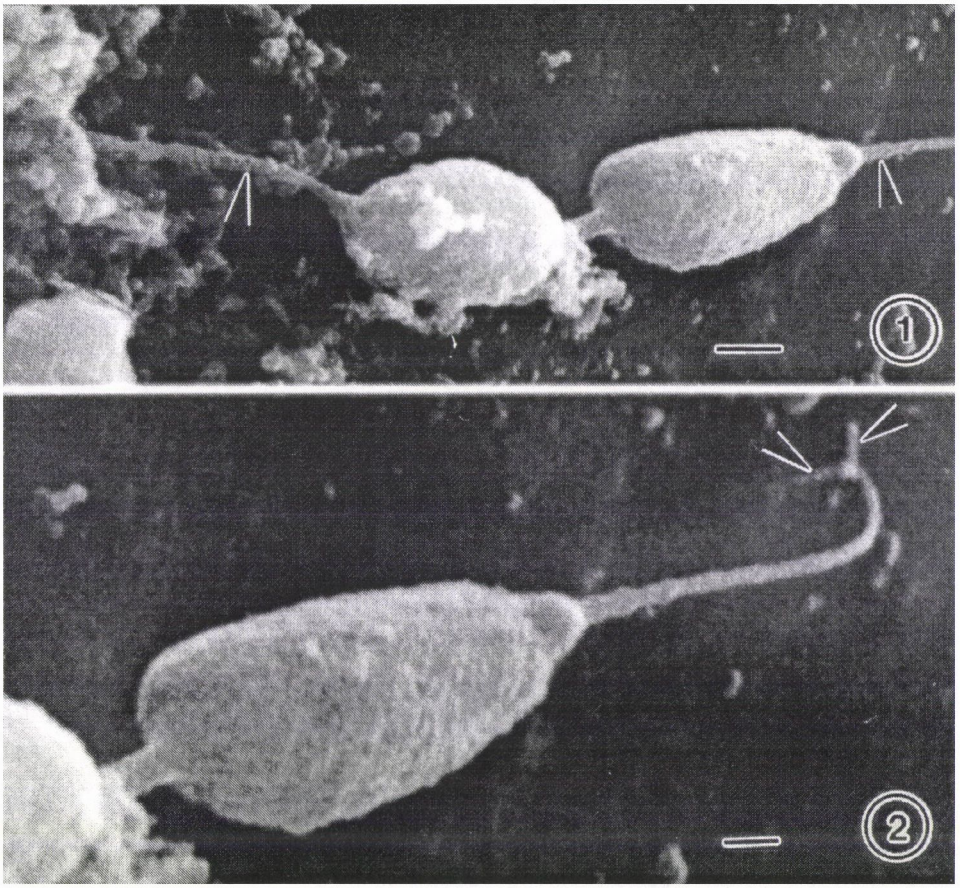
suggesting that microtubules are also involved in movement. The vegetative nucleus and generative cell, associated as they are in a male germ unit (MGU), appear to travel as a linked unit in the pollen tube (Russell and Cass, 1981; Dumas et al., 1984). This promotes their continued movement, maintaining a narrow distance from the elongating tip.

Based on studies using serial ultrathin sectioning and cellular reconstruction, a number of observations may be made about the origin, development and morphogenesis of flowering plant sperm cells and their progenitor generative cell. Generative cells are initiated as lenticular cells which peel from their external position and migrate into the pollen cell, assuming an elongated and often spindle-shaped form (Russell et al., 1996). One end of the generative cell is associated with a pre-existing groove on the surface of the vegetative nucleus, apparently held together in part by the complementarity of their surfaces. When the generative cell divides within the growing pollen tube, as in bicellular pollen, a constrained mitotic spindle is formed (Yu and Russell, 1993). Cytokinesis in the resulting two sperm cells is then initiated by a transverse, apparently callose-rich crosswall (Yu et al., 1992), which appears to be characteristic of tricellular pollen species as well (Russell et al., 1996). The region linking the two sperm cells is constricted.

The two sperm cells resulting from generative cell division may differ in their internal and external organization, displaying two distinct forms (dimorphic), or may have only one form (isomorphic). When sperm cells differ, they appear to do so consistently. Since only one of the sperm cells is typically associated with the vegetative nucleus, the sperm cells can be unambiguously designated S_{vn} (associated) or S_{ua} (unassociated). When dimorphism occurs, typically one of the two cell types is consistently larger (Russell, 1991). Sperm cells of tobacco typically have blunt tips, but some may have elongated projections of the cell at both poles of the MGU (Fig. 1). The sperm cell associated with the vegetative nucleus usually narrows to a slender elongated tip (Tian et al., 1998). The opposite end often ends in a blunt tip, but it may also be strongly elongated (arrowhead, Fig. 1). In unusual cases the projection may even bifurcate (arrowhead, Fig. 2).

Sperm cell dimorphism in flowering plants is most extreme in *Plumbago zeylanica,* where the organelle content differs greatly enough to have potential hereditary consequences. In *Plumbago*, one sperm cell contains almost all of the plastids (S_{ua}), whereas the other sperm (S_{vn}) usually contains no plastids and mitochondria are about five times as numerous (Russell, 1984). The S_{ua} – containing most of the plastids – preferentially fuses with the egg (>95%, Russell, 1985 and subsequent observations). This observation suggests that the sperm cells may differentiate in both structure and function, and that one of the cells may have an inherent advantage in competition to fuse with the egg cell (Russell, 1985).



Figures 1,2. Scanning electron micrographs of an isolated sperm cell of tobacco (*Nicotiana tabacum*). Fig. 1. Linked sperm cells with projections (arrowheads) on both ends of the cell. ×4000, Bar = 2 μm. Fig. 2. A higher magnification view of the presumed S_{vn} clearly indicates the presence of a bifurcation at the tip (arrowhead). ×6500, Bar = 1 μm

Tobacco sperm cells differ in shape (Yu et al., 1992), but are isomorphic with respect to organelle content and volume at maturity (Yu and Russell, 1994). According to quantitative three-dimensional reconstruction, sperm cells observed as late as 24 hr after germination of the pollen (approx. 24 hr before fertilization) are isomorphic, though the sperm cells have lost considerable volume (Yu and Russell, 1994). Strong evidence of cytoplasmic diminution was observed during this research, and it was noted that the S_{vn} appeared to be losing cytoplasmic volume at a greater rate than the other cell. Recent work suggests that cytoplasmic diminution of the S_{vn} does indeed result in that cell being significantly smaller than the unassociated sperm cell (S_{ua}). Ultimately, these differences become significant (unpublished data). These structural differences, if related to function, suggest the potential for preferential fertilization.

Tobacco is the most painstakingly studied example of "isomorphic" sperm cells, so the discovery that its sperm cells diverge in morphology just prior to fertilization casts doubt on the occurrence of isomorphic sperm cells in other plants. Perhaps in the isomorphic plants observed to date, the sperm cells were simply sampled prematurely. As further work is conducted, other isomorphic sperm cells may, upon further examination, display cellular differences underlying their morphological differences. Minor changes in the sperm cells accentuated during maturation could result in significant sperm cell differences, as in tobacco, that could have repercussions in sperm selection during fertilization.

Further studies of tobacco indicate that the fusigenic condition of the sperm cells changes with maturation. Newly formed sperm cells can be induced to fuse with each other relatively easily, whereas older sperm cells do not. This pattern can be reversed by using dilute concentrations of the enzymes cellulase and pectinase, suggesting that a carbohydrate periplasm surrounds the mature sperm cells and inhibits self-fusion (Tian and Russell, 1998).

Fertilization in flowering plants requires a detailed understanding of sperm cell biology (Russell, 1996). As additional work has been conducted, it has become evident that the sperm cells have a complete cellular physiology, with transcription, translation, their own gene expression patterns and complicated morphologies that promote their passage and ultimate fusion. The search for genetic mechanisms expressed differentially in paired sperm cells may ultimately answer the question of whether the two sperm cells of the successful pollen tube are truly interchangeable.

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Symposium paper

POLLEN-PISTIL INTERACTION: FACTS AND IMPLICATIONS*

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The phase that elapses from pollination to fertilization is marked by an intense male-female interaction. The mechanisms regulating this interaction are underpinned by the long journey that the pollen tube has to travel along the pistil until it reaches the female gamete. Several structures along this pathway interact with the growing male gametophytes, since the pistil, far from being a passive structure to support pollen tube growth, has an active role in regulating this growth. The reasons for this finely tuned interaction have to be sought in the resulting action of this process, the next generation. Here, the main events that occur along the pollen tube journey and how they interact with the pistil are examined. Finally, the possible implications of this interaction in the next generation are evaluated.

Key words: pollen, pollen tube, pistil

Introduction

In flowering plants, the reproductive process is far from straightforward and the time that elapses from pollination to fertilization is marked by an intense male-female interaction (Knox, 1984; Herrero, 1992a). As a result of this interaction, fertilization does or does not take place. While mate choice and courtship are well established in animals, this idea sounds extravagant in plants, where due to their sessile nature active mate choice cannot occur. However, maybe due to this sessile nature, plants have developed sophisticated mechanisms that regulate this male-female interaction. Some of them are well known, others are just emerging and others are to come. All of them appear to be underpinned by the long journey that the pollen tube has to travel along the pistil until it reaches the female gamete. Several structures along this pathway interact with the growing male gametophytes (Heslop-Harrison and Heslop-Harrison, 1985; Mascarenhas, 1993). This fascinating journey of a cell travelling in the middle of thousands of unknown cells to meet a unique hidden cell is further complicated by the fact that nourishment has to be driven along this journey to support the very active building of new pollen tube wall. This nourishment comes from the pistil. However, the pistil, far from being a passive structure to support pollen tube growth, has an active role in regulating this growth. In this regulation pistil development plays an important part and the maturation of the different pistilar structures occurs in an orchestrated way finely tuned to fulfil different functions. The question is, why should plants make a potentially simple thing so complicated. The answer perhaps

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has to be sought-as in courtship-in the next generation, since out of this interaction only a few genotypes survive and contribute to the next generation. Here, the main events that occur along the pollen tube journey and how they interact with the pistil are examined. Finally, the possible implications of this interaction in the next generation are evaluated.

The pollen journey

In angiosperms the female gamete is jealously wrapped in a number of successive envelopes that the pollen tube has to traverse to reach its target. Thus, along this journey the pollen tube grows through a varied landscape and faces a number of changing situations. When the pollen grain lands on a stigma, it hydrates and produces a pollen tube that carries the male gametes. Germination at the stigma occurs in an autotrophous way at the expense of the resources accumulated in the pollen grain (Herrero and Dickinson, 1981). Upon penetration into the style, the pollen tube grows close to the pistilar cells, either of the transmitting tissue, in species with a compact style, or of the stylar canal, in species with a hollow style. Early physiological work demonstrated that pollen tube growth in both hollow (Labarca and Loewus, 1973) and solid (Herrero and Dickinson, 1979) styles is heterotrophous, and at the expense of the stylar reserves. In the last few years research has moved fast to identify the molecules that are actively responsible for this interaction (reviewed in Cheung, 1996). Once the pollen tube has traversed the style it encounters the ovary. This area has been far less studied, perhaps due to the fact that structurally it is more complicated and that the key cells are hidden under several cell layers. In *Prunus*, pollen tube growth is not continuous and a number of accelerations and decelerations have been described. Upon arrival at the ovary, the pollen tube faces a placental protuberance, the obturator, where it stops for several days until this structure enters a secretory phase and pollen tube growth is resumed (Arbeloa and Herrero, 1987). Once the pollen tube has passed the obturator, growth is by no means straight and it further stops and wanders around before entering the ovule (Herrero and Arbeloa, 1989) and the nucellus. At these points while some pollen tubes are lost for ever (Herrero, 1992b), others resume growth and achieve fertilization.

The role of the pistil

While pollen life is marked by intense activity, the pistil is also in a state of continuous change. From an early view of the pistil as a passive supporting role, recent information shows that the pistil has an active part in controlling pollen tube growth (Herrero and Arbeloa, 1989; Herrero and Hormaza, 1996). It is clear that, upon flower development, the pistil has to attain a certain degree of development to support pollen tube growth (Kandasamy et al., 1994). However, this development does not cease upon flower opening and, on the contrary, the flower lifespan is marked by an intense continuous development. Maturation of the pistil occurs in a basipetal way, starting at the stigma and finishing at the ovule (Herrero

and Arbeloa, 1989). The stigma provides an adequate medium for pollen germination and recent work shows that the control of this hydration relies on the lipid surface that covers the stigma in wet stigmas and the pollen in dry stigmas (Wolter-Harts et al., 1998). Similarly the style provides nutritive support. Finally, growth in the ovary appears to be regulated by the different pistil structures the pollen tube has to traverse (Herrero, 1992b). Recent studies with defective mutants (reviewed by Drews et al., 1998) reveals that the embryo sac is required for successful pollen tube penetration and that this process is also subject to sporophytic control (Wihelmi and Preuss, 1996). The fact that the pollen tube relies on pistil nourishment, together with the fact that this nourishment is not given ad libitum but at discrete places and times (Herrero and Hormaza, 1996), stresses the importance of male-female synchronism for successful fertilization.

The next generation

The complexity of the male-female interaction contrasts with the fact that these mechanisms and the structures involved are highly conserved. This well preserved complexity suggests that it must confer an evolutionary advantage. Perhaps, as with courtship, the reasons for this finely tuned interaction have to be sought in the resulting action of this process, the next generation. Plants, as opposed to animals, do not have an independent germ line, and are subject to the accumulation of mutations in the reproductive cells. Pollen-pistil interaction surely provides an opportunity for selection against deleterious mutants (Chasan and Walbot, 1993). This phase also provides a framework for pollen-pistil incompatibility (Heslop-Harrison, 1983), enhancing outcrossing. But perhaps selection goes further and screening for other characters could well be occurring, promoting gametophytic selection (Mulcahy, 1979) and mate choice in plants (Marshall and Folsom, 1991). Indirect evidence from a number of unrelated species suggests that this might well be the case (Hormaza and Herrero, 1994). Indeed pollen-pistil interaction promotes a situation of competence for both male and female gametes out of which only a few survive up to the next generation. At the style, competition is mediated through the restricted support of pollen tube development. It has been shown in a number of species that, from the stigma to the ovary, the transmitting tissue has a funnel shape reducing both the area and the carbohydrates available for pollen tube growth (Herrero, 1992b; Herrero and Hormaza, 1996). Similarly, out of the pollen-pistil interaction that occurs in the ovary, only some ovules get fertilized. The mechanisms regulating this thinning have to be fully revealed, but it appears clear that only the ovules that are able to retain their starch are the ones that survive (Rodrigo and Herrero, 1998). Work is in progress to evaluate the influence of pistil developmental stages and carbohydrate reserves on reproductive success. For the time being it appears clear that the stigma and the style are well prepared to support pollen tube development but also to thin down the male gametophytic population. The ovary, in turn, appears as a domain well prepared to interact with and guide the pollen tube towards the female gamete. Interestingly, out of this interaction only some ovules become fertilized.

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Symposium paper

ARABIDOPSIS MUTANTS DEFECTIVE IN MALE GAMETOPHYTE DEVELOPMENT: A REVIEW*

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Within the past few years increasing numbers of laboratories have begun investigating male gametophyte development in *Arabidopsis thaliana* by the creation and study of mutants, and new knowledge is being generated at a rapid pace. Mutations at all stages of pollen development and in various anther and stamen cells and tissues have been described which result in male sterility. These mutants and the new information that they are providing is the subject of this review.

Key words: *Arabidopsis*, mutant, male gametophyte

Introduction

The small size of its genome, its short life cycle, and the relative ease of producing and characterizing mutants by the isolation of the genes involved, makes *Arabidopsis thaliana* an attractive plant for studying the genetic control of differentiation. The use of gene mutants to unravel mechanisms of development has been very powerful and successful in organisms such as *Drosophila* and *Caenorhabditis elegans* and is now increasingly being applied to study developmental problems in *Arabidopsis*.

After the identity of a flower primordium is specified in the floral meristem, the stamen primordium in the third whorl of the developing flower is initiated. The generation and use of genetic mutants in *Arabidopsis* and in other plants has been remarkably successful in elucidating the complex pathways of gene regulation involved in the making of a flower and in the control of stamen specification (Weigel and Meyerowitz, 1994; Yanofsky, 1995). Homeotic genes are involved in specifying stamen primordium initiation and that of other floral organs (Bowman et al., 1993). Interfering with several of the steps in floral development and especially in anther primordium initiation will result in male-sterile plants. The topic of stamen initiation, however, is excluded from this review. Floral organ specification has been reviewed recently (Meyerowitz, 1998).

After the anther primordium is formed, the morphology of the anther is established and cell and tissue differentiation occur. A number of stages have been identified in stamen development (Goldberg et al., 1993), a defect in any one of which could result in male sterility. In brief, within the sporogenous tissue in the anther locules, pollen mother cells differentiate and undergo meiosis, forming

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tetrads of haploid microspores. The cells of the tapetum which surround the sporogenous cells are intimately involved in pollen development. The microspores enlarge and undergo biochemical and morphological changes. An unequal mitotic division, microspore mitosis, produces two cells, the vegetative cell and the generative cell, in the young pollen grain. The generative cell will divide, producing two sperm cells which later function in double fertilization. On pollen maturation, the anther dehisces, releasing the pollen. The last stage of development is germination of the pollen grain on the stigma, production of a pollen tube which grows through the female tissues of the pistil and conducts the sperm cells to the embryo sac, and finally the actual fusion of the two sperm cells, one with the egg and the other with the central cell.

Horner and Palmer (1995) reported from a review of the literature that about 60 genes have been described that are known to affect male fertility in maize, and a similar number have been found for rice and tomato. Of the described mutations, approximately 14% affect sporogenous cells before meiosis, about 47% affect meiosis and tetrads, about 29% affect various stages of microspore development and about 10% cause defects in bicellular and later pollen stages (Horner and Palmer, 1995). It has been estimated that transcripts of about 20,000 different genes are found in the mature pollen grain of *Tradescantia paludosa* and maize, of which about 10% might be unique to pollen (Willing and Mascarenhas, 1984; Willing et al., 1988). One might thus expect many more than 60 genes to affect male fertility and the unique properties of *Arabidopsis* make it likely that these additional genes will be identified and characterized in this plant.

The *Arabidopsis* mutants are discussed according to the stage in pollen development where the genes act.

Mutants in meiosis

A number of mutants have been described in *Arabidopsis* that exhibit abnormalities in meiosis (Chaudhury et al., 1992, 1994; Chaudhury, 1993; Dawson et al., 1993; Glover et al., 1990; He et al., 1996; Peirson et al., 1996; Hulskamp et al., 1997). Male premeiotic and meiotic development are specified by genes expressed in the sporophyte. Sporophytic genes can also affect post-meiotic microspore development (Taylor et al., 1998).

Dawson et al. (1993) studied five non-allelic recessive male-sterile mutants produced by seed mutagenesis with X-rays and ethyl methanesulphonate (EMS). In plants homozygous for the *msY*, *msW* and *msK* mutations, pollen development became abnormal at or before meiosis, whereas in *ms1*, development was arrested immediately after microspore release from tetrads. The *MSI* gene has been mapped to the top arm of chromosome 5 (Thorlby et al., 1997). In *msY*, pollen mother cells (PMCs) were abnormally granular prior to the first division of meiosis and formed abnormal tetrads with callose mostly absent. Meiotic products and tapetal cells in *msY* degenerated. In *msW* plants, PMCs were abnormal, meiotic products were irregularly shaped and normal tetrads were absent. Tapetal cells and the products of meiosis became vacuolated and degenerated (Dawson et al., 1993). In the *msK* mutant, PMCs and tetrad-like cell groups had abnormal cytoplasm and callose and the timing of callose dissolution was aberrant.

Four nuclear male-sterile mutants produced by chemical mutagenesis (EMS) were described by Chaudhury et al. (1994). All four mutants showed premeiotic lesions affecting different stages of development. The tapetum and middle layer of the anther wall are abnormal in *ms3* and PMCs degenerate. In *ms4*, meiotic division is impaired and dyads persist in the anther. Degeneration of all anther cells occurs early in development in *ms5*, although the PMCs initially appear normal. In the *msl5* mutant, the tapetum and PMCs degenerate early in anther development.

Hulskamp et al. (1997) described mutations in the *STUD (STD)* gene in lines produced by EMS mutagenesis. The *STUD* gene is needed for male-specific cytokinesis after telophase II of meiosis. Normal meiotic nuclear divisions occur in *std* anthers, but no cell walls form and the resulting single microspore is tetranucleate. Further development is apparently normal and results in a large pollen grain which contains four vegetative nuclei and as many as eight sperm cells. The enlarged pollen grains produce a single pollen tube and *std* mutants are partially male fertile.

Four mutant alleles of the *TETRASPORE* (*TES*) gene have been isolated (Spielman et al., 1997). The primary defect in *tes* mutants is failure of cytokinesis in male meiosis. This mutant is very similar if not identical to mutants in the *STUD* gene (Hulskamp et al., 1997). In *tes* mutants, abnormalities such as fusion of nuclei, formation of internal walls and abnormal external wall patterning have been described (Spielman et al., 1997).

Preuss et al. (1994) described a very interesting and potentially useful mutant, *QUARTET (QRT).* Two genes, *QRTI* and *QRT2*, are required for normal pollen separation from tetrads. In *qrt* mutants, parts of the outer walls of the four microspores in a tetrad remain fused and the pollen grains develop in groups of four: all pollen grains in the tetrad are viable. These mutants make it possible to perform tetrad analysis and they have been used in crosses to confirm the gametophytic expression of the *sidecar pollen* gene (Chen and McCormick, 1996). The *qrt* mutant has also been useful in allowing a better characterization of the abnormal microspores formed in the *mei1* mutation because the microspores are held together after tetrad dissolution (He and Mascarenhas, 1998). There are pectic components in the primary wall of the pollen mother cell which surrounds the callose wall around the pollen mother cell and later the tetrad. In wild-type pollen the pectic components are no longer detectable at the time of microspore release from tetrads. In *qrt1* and *qrt2* mutants these pectic components appear to persist in the wall after callose dissolution (Rhee and Somerville, 1998). The *QRT1* and *QRT2* genes may thus be required for the cell-type-specific degradation of pectin that is necessary for the normal separation of individual microspores from tetrads.

Male-sterile *Arabidopsis* lines produced by insertional mutagenesis using seed transformation have been generated by Feldmann (1991). The availability of these lines, which were initially identified by a screening carried out by Robert Goldberg, has provided a means to isolate and characterize genes important in pollen development. Glover et al. (1996) performed a DNA blot

analysis of 23 of Feldmann's reduced-fertility lines. Mutants that were male-sterile and mutants that showed both male and female sterility were identified. Forty percent of the lines studied showed a single locus of insertion of the T-DNA; the rest segregated for two or more T-DNA inserts. Six lines were studied for the linkage of T-DNA inserts and mutant phenotype. The mutation segregated with a T-DNA insert in three lines (178, 1926, 2522), whereas in the other three lines (1180, 1885, 2379) the T-DNA and mutant phenotype were not linked.

Peirson et al. (1996) described at a cytological level three Feldmann-generated male sterile mutants which exhibit defects in meiosis. All three mutants are normal until the onset of meiosis. In line 6492 as many as eight microspores of variable size are seen in each callose-enclosed tetrad. The other two mutants (7219 and 7593) are similar in many respects showing asynchronous and variable development, lack of callose production, and production of coenocytic cells. Mutant 7593 is female-fertile while mutant 7219 is female-sterile. The three mutants are T-DNA tagged mutants and this should facilitate isolation of the genes involved.

A more detailed cytochemical analysis of the previously studied (Peirson et al., 1996) mutant line 6492, which is now referred to as *syn1*, has been made (Peirson et al., 1997). The first noticeable defect is seen at telophase I. Some chromosomes seem scattered in the cytoplasm but attached to stray microtubules. It is suggested that *syn1* is defective in the formation of the synaptonemal complex and/or cohesion between sister chromatids.

A T-DNA tagged mutant, *mei1* (Feldmann line 2522), defective in meiosis and producing a "tetrad" consisting of from five to eight microspores of varying size and DNA content, has been described (He et al., 1996). The microspores are released from the tetrads, but degenerate. This mutant is female fertile. Plant DNA flanking the inserted T-DNA was isolated by inverse PCR and a genomic fragment spanning the T-DNA insertion site was isolated from a wild-type genomic library (He and Mascarenhas, 1998). Using RT-PCR and RNA isolated from very young flower buds, a cDNA fragment was obtained. Nucleotide sequence comparison of the cDNA and the genomic sequence in this region indicated a gene which contained two introns. The deduced sequence of the *MEI1* gene product, which contains 89 amino acids, shows possible similarity with the human acrosin-trypsin inhibitor, HUSI-II. Two partially overlapping genomic DNA fragments, one containing the entire *MEI1* gene and the other containing part of the 3' end of the gene, were used to transform mutant *mei1* plants. The fragment containing *MEI1* showed complementation of the mutant phenotype, confirming that *MEI1* is the gene involved (He and Mascarenhas, 1998). Whether the similarity of *MEI1* to Kazal-type inhibitors is meaningful is not clear at present. It is tempting, however, to speculate, since regulated proteolysis performs a very important role in cell division (Hoyt, 1997), that the *MEI1* protein might be involved in essential degradative processes during meiosis, and that its absence results in the *mei1* phenotype.

Microspore and early pollen mutants

Mutants deficient in adenine phosphoribosyltransferase (APRT) activity were obtained after EMS mutagenesis. The mutants are male sterile, with abortion occurring after release of microspores from the tetrads (Moffatt and Somerville, 1988; Regan and Moffatt, 1990). In the *apt1* mutant, which is male sterile (Gaillard et al., 1998), the metabolism of cytokinins is impaired (Moffatt et al., 1991). APRT is the major enzyme in plants for the conversion of adenine to AMP and is probably the predominant pathway for the salvage of adenine (Moffatt et al., 1991). It is, however, not yet known whether male sterility is caused by a defect in adenine salvage or in the cytokinin metabolism. The gene for *APT1* has been genetically mapped to chromosome 1, and has been cloned (Moffatt et al., 1992, 1994).

Taylor et al. (1998) described seven new male-sterile mutants (*ms7–ms13*) that show post-meiotic defects in microspore development. Microspores released from tetrads are irregular in shape and often lack exines in the *ms9* mutant. In *ms12* mutants, microspores lack normal exine sculpturing and degenerate, indicating that the *MS12* gene product is probably involved in exine formation. The tapetal cytoplasm breaks down in *ms7* mutant anthers. In *ms8* mutants, intine development is damaged although the exine is normal, and pollen grains rupture. The first detectable aberration in *ms11* mutant plants occurs at the mid- to late-vacuolate stage in the microspore. The microspores and tapetal cells appear to have lost their DNA.

The *Arabidopsis MALE STERILITY 2* gene has been studied in mutant lines obtained by *EnlSpm-IldSpm* transposon tagging (Aarts et al., 1993, 1997). The first aberrations in pollen development are seen in *ms2* mutants when microspores are released from tetrads. The tapetum has larger cells than normal and the released microspores have irregular shapes and do not seem to produce an exine. The microspores and tapetal cells eventually degrade, although occasional pollen grains with very thin walls lacking an exine persist and seem to be responsible for the small amount of seed produced in plants homozygous for the mutation (Aarts et al., 1997). The *MS2* gene has been cloned and shows similarity to a fatty acid reductase from jojoba (JJFAR). This protein is involved in synthesizing storage wax esters in developing jojoba seeds. It is likely that the *MS2* gene is involved in the reduction of fatty acyl groups to fatty alcohols in the formation of sporopollenin (Aarts et al., 1997). *In situ* RNA hybridizations show that the expression of *MS2* is located specifically in wild-type tapetal cells at the stage when microspores are released from tetrads, before any significant pollen exine has formed. *MS2* mRNA is not detectable when the exine wall has thickened. This correlates well with the first appearance of aberrations in *ms2* anthers. The *MS2* promoter was coupled to a *GUS* gene and introduced into tobacco. *GUS* activity was restricted to the tapetum in anthers at the time of release of microspores from tetrads. Interestingly and unexpectedly, however, the promoter was active in mature pollen. Whether this is real or an artifact of

the transformation is not clear at present (Aarts et al., 1997). These results are very informative because they demonstrate that very early in microspore development gene products of the tapetum have significant roles to play in pollen wall formation and probably in other events as well.

The only male gametophytic mutant that has been described in *Arabidopsis* is *sidecar pollen* (Chen and McCormick, 1996). The *SIDECAR POLLEN* gene is required for normal cell divisions in the developing pollen grain. In the mutant, a significant fraction of the pollen grains has an extra cell with a single diffuse nucleus which seems to be a vegetative cell. The extra vegetative cell in *scp* pollen is separated from the normal three-celled male gametophyte by an intine wall, although the two are enclosed within the same exine wall. The extra cell is produced before the asymmetric microspore mitosis in the "normal" cell. In *scp* microspores the first division results in two cells of almost equal size. The mutant *scp* gene affects the viability of pollen in addition to the number of cell divisions. Normal pollen, aborted pollen, and pollen with an extra cell are produced in *scp* mutants and the proportion of the three types of pollen varies depending on the genetic background of the plants. In the background of the Landsberg *erecta* ecotype, for example, *scp* is a gametophytic lethal. The extra cell and the normal gametophyte are both capable of producing tubes.

Mutations affecting events late during pollen development and during pollination

Several *Arabidopsis* mutants have been identified which affect the interaction of pollen grains with stigma cells. The *pop1* (*cer6-2*) mutant (defective in pollen pistil interactions) produces morphologically normal pollen, but the mutant pollen fails to germinate on the stigma (Preuss et al., 1993). Mutant pollen does not absorb water from the stigma but germinates in vitro. The *pop1* mutant lacks stem waxes and is similar in phenotype to several wax-defective mutants, *ECERIFERUM (CER)*, that also show reduced fertility (Koorneef et al., 1989). Complementation analysis has shown that *pop1* is allelic to *cer6-1* and hence is now called *cer6-2* (Preuss et al., 1993). The infertility of *cer6-2* pollen on the stigma is reversed when the mutant plants are grown in a high humidity environment or when the pollen is mixed with wild-type pollen. The stigma surface is apparently able to discriminate among pollen grains, with wild-type grains being hydrated while *cer6-2* pollen is not. Wild type pollen contains several long chain (29 and 30 carbon) lipid compounds, but these lipids are almost absent from *cer6-2* pollen (Preuss et al., 1993). The tryphine coating is essentially absent in *cer6-2* pollen walls and pollen from other cer mutants contains tryphine which has smaller and fewer lipid droplets than wild-type pollen.

Three mutants allelic to *cer1, cer3* and *cer6* have been isolated independently (Hulskamp et al., 1995). The block in the recognition of pollen by stigma cells in *cer* mutants can be suppressed by pollination at lower temperatures in addition to high humidity (Hulskamp et al., 1995).

The *CER1* gene was isolated after gene tagging with the heterologous maize *Enhancer Inhibitor* transposable element system (Aarts et al., 1995). It encodes a protein that seems to be involved in the conversion of aldehydes to alkanes and is likely an aldehyde decarbonylase. The *CER1* gene is transcribed in wild-type stem and fruit tissue and also in flowers. Observations of Arts et al. (1995) support the hypothesis of Preuss et al: (1993) that long chain lipids, particularly alkanes, are important in the tryphine layer for proper pollen-pistil interactions.

The first indication that signalling by the phytohormone methyl jasmonate might be involved in pollen development came from a study of the *coi1* mutation (Feys et al., 1994). The *coi1* plants are resistant to coronatine, a phytotoxin produced by a bacterial pathogen; they are insensitive to methyl jasmonate, and they are also male sterile. Stamens of *coi1* plants have shorter filaments than wild-type plants. The anthers which do not dehisce contain infertile pollen grains with conspicuous vacuoles. Coronatine and jasmonate seem to affect the same response pathway and possibly act on the same receptor (Feys et al., 1994).

In *Arabidopsis*, at least three desaturase enzymes are involved in fatty acid synthesis in the conversion of 18:2 and 16:2 acyl groups to 18:3 and 16:3. The fatty acid desaturation (*FAD*) genes, *FAD7* and *FAD8*, are two chloroplast isozymes, and the *FAD3* gene product is localized primarily in the endoplasmic reticulum. McConn and Browse (1996) produced a *fad3-2 fad 7-2 fad8* triple mutant line which contained less than 0.1% trienoic fatty acids. These plants showed wild-type growth and vegetative development but were male sterile. In the mutant, pollen grains developed to the tricellular stage. Fertility was restored in the mutant by the exogenous application of α-linolenic acid or jasmonate to flower buds 12–24 hours before flower opening. Jasmonic acid is synthesized from 18:3 Iipids. These results provide strong evidence for a critical role for jasmonate in the terminal stages of pollen development (McConn and Browse, 1996).

The *cpd* mutation inhibits cell elongation that is controlled by the brassinosteroid hormone, brassinolide (Szekeres et al., 1996). *cpd* plants have severe pleiotropic effects, including male sterility, because pollen does not germinate. Unfortunately the exact stage during pollen development when the defect is first seen was not reported. The *CPD* gene was isolated by T-DNA tagging and encodes a cytochrome P450 which shares homologous domains with steroid hydroxyases. Brassinolide treatment restored the *cpd* mutant plants to wild-type, including restoration of male fertility (Szekeres et al., 1996). Because of the pleiotropic effects of the *cpd* mutation it is unclear whether brassinosteroids are directly involved in pollen development or whether male sterility is an indirect effect of the mutation. Brassinosteroids, however, are abundant in pollen (Clouse and Sasse, 1998).

Evidence has been found for a critical role for flavonoids in pollen germination in maize and petunia (Taylor and Jorgensen, 1992; van der Meer et al., 1992; Ylstra et al., 1994). Addition of flavonols to pollen, anthers or stigmas of mutant plants which lack chalcone synthase (CHS) activity results in recovery

of pollen germination (Mo et al:, 1992;Ylstra et al., 1994). Surprisingly, however, *Arabidopsis* mutants completely deficient in flavonoid biosynthesis are fully male fertile (Burbulis et al., 1996). These results indicate that although high levels of flavonoids are present in pollen of most species, they are not universally required for fertility, raising interesting questions about the functional evolution of these compounds.

Two genes, *POP2* and *POP3*, have been described that affect pollen tube guidance in the ovule and are important for self fertility (Wilhelmi and Preuss, 1996). Only when male and female tissues were defective in both genes did self-sterility occur. In the sterile mutant, pollen tubes in self-pollinated pistils did not grow towards the micropyle. They grew in a random manner in the ovary locule whether or not they were in proximity to the micropyle. Mutant pollen tubes did not adhere to pistil cells. Genetic tests indicated that both genes are sporophytically expressed. This is somewhat unexpected because of the late appearance of the lesion, but it shows similarity to sporophytic self-incompatibility.

Mutations in non-pollen tissues

Interfering with events in non-pollen tissues in the stamen can result in male sterility. In the *msH* mutant, for example, anther dehiscence is blocked and in the *msZ* mutant elongation of the anther filament is blocked. Plants with both these mutant genes are male sterile (Dawson et al., 1993). In the *por1-1* mutation, which is T-DNA tagged, stamen filaments are shorter than wild-type and anther dehiscence is delayed (Park et al., 1996). In this mutant, however, pollen grains are round instead of tricolpate, and some mutant pollen grains have abnormal surface coatings and do not germinate either *in vitro* or on the stigma. The primary defect in *por1-1* is thus likely to occur during later pollen development in the anther. The pleiotropic effects of this mutation might, moreover, indicate multiple sites of activity both in the sporophyte and in the gametophyte.

Concluding remarks

Mutants provide an estimate of the number of important genes involved in specifying the development of the male gametophyte so that it can carry out its essential functions. A large number of mutants have been created in *Arabidopsis* by chemical mutagenesis and also by insertional mutagenesis and a beginning has been made in isolating the genes responsible. Mutants have already provided new information about pollen development that might not have been otherwise possible. The identification, for example, of jasmonate and brassinolides as important to pollen development was made possible by the isolation of mutants. The pace of gene isolation is anticipated to accelerate in the next few years and can be expected to result in greatly increased understanding of pollen development. A fairly large number of the mutants described are involved in

meiosis and are sporophytically expressed. Only one mutant, *sidecar pollen*, has thus far been characterized which is gametophytic in expression. No nuclear regulatory genes have yet been described, but the *SIDECAR POLLEN*, and the *STUD* and *TETRASPORE* genes are probably good candidates. The identification and isolation of regulatory genes should be a high priority because such genes will elucidate the critical events that regulate male gametophyte development.

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Symposium paper

THE MOLECULAR MECHANISM OF SELF-INCOMPATIBILITY IN *BRASSICA*: RECENT ADVANCES

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REPRODUCTION ET DÉVELOPPEMENT DES PLANTES, UMR 9938 CNRS-INRA-ENSL, ECOLE NORMALE SUPÉRIEURE DE LYON, 46 ALLÉE D'ITALIE, 69364 LYON CEDEX 07, FRANCE
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The last few years have seen several significant advances in our understanding of the self-incompatibility (SI) response in *Brassica*. The stigma-specific expression patterns of two *S* (self-incompatibility) locus linked genes, *SRK* and *SLG*, have been studied in detail. Both *SRK* and *SLG* have been shown to encode more than one protein product and there is evidence for a correlation between the spectrum of proteins present at the stigma surface and the strength of the SI response. Sequence analysis of multiple alleles of *SLG* and *SRK* has provided information about their evolution and suggests that the specificity of different *S* alleles is determined in a complex manner. Two important advances have been made in the last year. Firstly, proteins have been identified which interact with the kinase domain of SRK, the *S* locus receptor kinase, and which are therefore potential downstream components of SI signal transduction. Secondly, the *MOD* gene which appears to be essential for the SI response has been shown to be very closely linked, and probably identical to, a water channel gene providing a possible connection between the SI response and pollen hydration. A combination of genetic and biochemical approaches is being used to identify the male component of SI, a putative ligand for SRK.

Key words: *Brassica*, multiple allele, self-incompatibility, SRK, SLG

Introduction

Many higher plant species possess a self-incompatibility (SI) system which allows the recognition and rejection of self-pollen grains and hence prevention of self-fertilisation (deNettancourt, 1977). The evolution of these self-incompatibility systems is thought to have made an important contribution to the success of angiosperm species because they prevent inbreeding and promote an exchange of genetic information between different members of a species. From an agricultural point of view, self-incompatibility can be a useful tool for the breeder, for example in the production of hybrid seed. Self-incompatibility is also important because it creates a barrier to self-fertility in many species that are grown for their seed or fruit.

Several different SI systems with markedly different genetic bases have been described. The genetic differences between the SI systems indicate that they have arisen independently and this conclusion is supported by recent molecular analyses of the genes controlling SI in the different systems. SI systems have been

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classified as either heteromorphic, where the presence of different *S* (for self-incompatibility) alleles is associated with morphological differences in the flower, or as homomorphic where there is no characteristic morphological difference between plants carrying different *S* alleles. Homomorphic SI systems can be either gametophytic (as for example in the *Solanaceae*) or sporophytic (as for example in the *Brassicaceae*) depending on whether the SI phenotype of the pollen is determined by its haploid genome or by the diploid genome of the male parent, respectively (McCubbin and Kao, 1996). In most cases SI is controlled by a single genetic locus, although systems have been identified where SI is controlled by two or more loci, for example the two locus system (S and Z) found in the grass *Phalaris coerulescens*. In recent years, significant advances have been made in our understanding of the mechanisms of self-incompatibility in studies which have concentrated on a limited number of model systems (McCormick, 1998). This review will present recent data concerning one of these model systems, the homomorphic, sporophytic self-incompatibility system found in species of the genus *Brassica*.

Self-incompatibility in *Brassica*

In *Brassica* ssp. SI is controlled by a single, highly-polymorphic locus, the *S* locus. Genetic evidence indicates that each allele encodes both a male and a female component and that it is the specific interaction of two components encoded by the same allele (or haplotype) which initiates the SI response.

SI in *Brassica* is sporophytic: the pollen SI phenotype is determined by the genotype of the diploid, sporophytic male parent. This indicates that pollen grains acquire SI determinants from the diploid cells of the anther but it is not known exactly how this happens in practice. Pollen development begins with a meiotic division of a pollen mother cell to produce a tetrad of microspores which will separate and undergo two subsequent mitotic divisions to produce the mature pollen grains. One possible explanation of the sporophytic nature of SI in this genus is that the *S* locus genes are expressed before meiosis, for instance in the pollen mother cell, and that the SI determinants are inherited by the developing pollen grains. Alternatively, the SI determinants may be expressed in other diploid tissues of the anther and transferred to the microspores during development. This second model is attractive because the tapetal layer of sporophytic cells which surround the developing microspores in the locule has been shown to break down during microspore development, releasing its contents onto the surface of the developing pollen grains.

Following dehiscence of the anther, the pollen grains must migrate to the stigmatic surface of the female part of the flower, the pistil, in order to complete the life cycle. This transfer is usually mediated by insect vectors. Several different processes then occur on the stigma surface. Firstly, there is an interaction between the pollen grain and the papillar cells of the stigma surface which allows the pollen grain to adhere strongly to the stigma surface. During this process, the pollen coat, which is the external layer of the pollen grain derived from material deposited by the tapetal cells, is modified where it comes

into contact with the papillar cell. Following adhesion, water is transferred from the stigma to compatible pollen grains so that they are able to hydrate. The hydrated pollen grains then germinate and emit a pollen tube which penetrates the stigma and grows down through the style to the ovaries at the base of the pistil. Following penetration of the pollen tube into the ovule, the two male gametes are deposited in the embryo sac where they fuse with the oocyte and the central cell to complete the process of fertilisation.

In *Brassica* the SI response occurs whilst the pollen grain is on the stigma surface. The earliest step, pollen adhesion, is not affected by the self-incompatibility response. Luu et al. (1997a) were unable to detect a difference between adhesion of compatible and incompatible pollen grains using a sensitive method to test the force of pollen adhesion. The fertilisation process is, therefore, blocked after the adhesion step but the precise stage at which the blockage occurs is variable depending, at least in part, on the genotype of the parent plants. Self-incompatible pollen may either 1) fail to hydrate, 2) hydrate but fail to germinate or 3) the pollen grain may germinate but the pollen tube fail to penetrate the stigma surface.

Although all the components which determine *S* allele specificity are predicted to be encoded by genes at the *S* locus, factors which affect other steps of the process or which regulate the expression of *S* alleles in a general manner may not necessarily be encoded by genes at the *S* locus. It has been known for some years that mutations in so-called modifier or suppresser genes can render self-incompatible plants self-compatible (Nasrallah, 1989). A molecular analysis of one of these loci is described below. Some of the more subtle influences of genetic background on SI were revealed in a recent study which monitored the evolution of the SI response over time in flowers of a group of plants which were all homozygous for the S_{15} haplotype but with different genetic backgrounds (Ruffio-Châble, 1998). The strength of the SI response was shown to vary considerably between the different plants. When the most self-incompatible plants were considered the rejection of self-pollen either remained strong right up until flower senescence or the SI response weakened in older flowers allowing a significant level of seed set. This study highlights one of the problems which can be encountered when SI is used as a breeding tool and suggests that future research should aim at a better understanding of unlinked loci which influence the SI response.

Molecular mechanism of the SI response in *Brassica*

Efforts to understand the molecular mechanism of the SI response in *Brassica* have concentrated on identifying genes located at the *S* locus and on characterising their role in the SI response. The first *S* locus gene to be identified was *SLG* which encodes the *S* locus glycoprotein, a secreted protein that is expressed abundantly in stigmas (Nasrallah *et al.*, 1987). A second *S* locus gene, *SRK*, shares sequence similarity with *SLG*. *SRK* encodes a membrane-localised protein which resembles animal receptor kinases (Stein et al., 1991; Delorme et al., 1995). The extracellular domain of SRK, which

closely resembles SLG, is separated from a cytosolic domain with serine/threonine protein kinase activity by a single membrane-spanning domain. Apart from their location at the *S* locus, there are several other reasons for believing that *SLG* and *SRK* play a role in the SI response. Both *SLG* and *SRK* are expressed specifically in the stigma, the site of the SI response (although both genes are also weakly expressed in anthers) and both genes are highly polymorphic. Alleles of both *SLG* and *SRK* can be divided into two classes based on their sequences and the degree of sequence polymorphism. These two classes correspond closely with the two classes of *S* haplotypes defined on the basis of phenotype, class I haplotypes giving a strong SI response and tending to be dominant whilst class II tend to confer a weak SI response and to be recessive to class I haplotypes. Finally, although their roles have not been demonstrated directly, there is genetic evidence that both *SLG* and *SRK* are important for the SI response (Toriyama et al., 1991; Nasrallah et al., 1994; Stahl et al., 1998).

The protein domain which is found in both SRK and SLG and which corresponds to the extracellular domain of SRK and the entire mature SLG protein, has been referred to as the *S* domain. No function has yet been assigned to this domain apart from its implied recognition function in the SI response but a number of other genes are known to encode proteins containing similar *S* protein domains. These genes, which have been grouped together in the *S* gene family, encode either secreted glycoproteins, similar to SLG, or receptor-like kinases similar to SRK. One member of this family, the *Brassica S* locus-related 1 (*SLR1*) gene encodes a secreted glycoprotein which, like SLG, is expressed specifically in stigmas. *SLR1* is not linked to the *S* locus and the secreted glycoprotein does not, therefore, play a role in the recognition step of the SI response. However, recent evidence indicates that the SLR1 protein contributes to the process of pollen adhesion (Luu et al., 1997b). It is interesting to speculate, therefore, that the SI system in *Brassica* may have evolved by adaptation of a molecule which was originally involved in the compatible pollination process. Alternatively, another member of the *S* gene family has been implicated in the defence response (Pastuglia et al., 1997a) and it has been suggested, based on the many similarities between pollen/pistil and plant/pathogen interactions, that SI may have evolved from a recognition system originally involved in plant defence.

Alternative splicing of SLG and SRK transcripts and the diversity of stigma S proteins

Both *SLG* and *SRK* are complex genes encoding multiple transcripts. For example, at least seven different transcripts are encoded by the class I SRK_3 gene, including transcripts from both strands of the DNA (Delorme et al., 1995; Cock et al., 1997). These transcripts code for at least two different protein products, the full-length SRK protein and a truncated, secreted protein, eSRK, which resembles SLG (Giranton et al., 1995). Similarly, two of the class II alleles have been shown to possess *SLG* genes with two exon sequences.

Alternative transcripts of these genes encode either SLG or a membrane-anchored form of SLG called mSLG (Tantikanjana et al., 1993). The function of these alternative forms of SLG and SRK are not known but it is thought, by analogy to truncated forms of receptor kinases in animal systems, that proteins such as eSRK and mSLG may modulate the receptor kinase activity of SRK. It is interesting in this respect that the *S* locus-encoded proteins present in stigmas of class II haplotypes differ from those present in class I haplotypes suggesting a correlation between the spectrum of proteins at the stigma surface and the strength of the SI response.

S haplotype specificity

SI systems, such as that found in *Brassica*, which are controlled by a single, highly polymorphic locus, are considered to be more highly evolved than, for example, heteromorphic SI systems found in other plants. A key requirement for the evolution of SI systems of this type is the development of a large number of alleles, each of which is able to function as a specific recognition system. On a molecular level, this means that as each allele evolved, the components of the recognition system acquired a new specificity similar to, but different from, that of its ancestor. Sequence information has now been obtained for a large number of alleles of the *SLG* and *SRK* genes and these sequences have been compared in an effort to determine how allelic specificity is determined. Both *SLG* and *SRK* alleles are polymorphic throughout their *S* domains although polymorphisms are more frequent in two variable domains on each side of a region containing 12 cysteines. The first of these two variable domains contains two regions which exhibit a particularly high polymorphism and have been designated hypervariable domains. Because of the concentration of polymorphism in these two regions, it has been suggested that the hypervariable domains may determine allelic specificity. However, a recent study has shown that *SLG* alleles can have identical hypervariable domains but be associated with different SI phenotypes (Kusaba et al., 1997). If SLG is one of the proteins that determines specificity in the SI response, then this observation suggests that specificity is not determined solely by the hypervariable domains but that there is probably a contribution from other regions of the molecule. It is likely that a real understanding of what determines the specificity of SI recognition will only be obtained when the male component is identified and some information is available concerning its interaction with the female component on a three dimensional level.

The male component of the SI response: a ligand recognised by SRK?

The male component of the SI response is predicted to be encoded by a sporophytically-expressed gene located at the *S* locus. One approach that is being used to identify this gene is to characterise genes which are physically linked to *SLG/SRK* at the *S* locus. These studies have shown that the *S* locus is a complex locus, including a number of different genes, and that, in some

haplotypes, the locus can extend over several hundred kilobases of the genome (Boyes et al., 1997). One of the genes identified by this approach, the *S* locus anther gene (*SLA*), fulfils many of the criteria for a gene encoding the male component (Boyes and Nasrallah, 1995). Apart from its location at the *S* locus, *SLA* is expressed specifically in anthers, is polymorphic in different *S* haplotypes and is predicted to encode a small peptide. A more recent analysis, however, has identified lines which are self-incompatible despite the fact that their *SLA* gene is interrupted by a retrotransposon-like sequence (Pastuglia et al., 1997b). This observation indicates that *SLA* is not required for the SI response. Several other genes have been identified at the *S* locus but they do not show the pattern of expression or degree of polymorphism expected for a gene involved in SI recognition and are thought to carry out other functions in the plant.

In an alternative approach to identifying the male component, Stephenson et al. (1997) have recently shown that proteins isolated from the surface layer of pollen, the pollen coat, can influence the SI response when applied to stigmatic papillar cells before pollination in an *in vitro* bioassay. Furthermore, a partially-purified, active fraction of the pollen coat proteins contained the PCP-A protein which has been shown to interact with SLG *in vitro* (although no allelic specificity has yet been demonstrated for this interaction). Location of the active fraction in the tapetum-derived pollen coat supports the model in which the male component is derived from the diploid cells of the tapetum.

Signalling via SRK: downstream targets

As mentioned above, the similarity of SRK with animal receptor kinases suggests that the SI response in *Brassica* is initiated by binding of a ligand to SRK acting as a cell-surface receptor and that the response may be mediated by phosphorylation of downstream substrates. Experiments using the yeast two hybrid system and/or *in vitro* binding assays have shown that the kinase domain of SRK can bind to a number of different proteins. These include ARC1 (a *Brassica* protein of unknown function which contains arm repeats similar to those found in the *Drosophila armadillo* protein and ß-catenin; Gu et al., 1998), two *Brassica* thioredoxin proteins (Bower et al., 1996) and the *Arabidopsis* kinase-associated protein phosphatase (KAPP; Braun et al., 1997). ARC1 is the most interesting of these proteins because it is expressed specifically in stigmas and because it associates only with the phosphorylated form of the SRK kinase domain. It is not clear for the moment how these proteins might be involved in mediating the SI response.

Very little is known about the downstream targets of the SI response and how they effect the inhibition of pollen germination. Pollen hydration seems to be an important step that is blocked in self-incompatible pollinations and there is evidence that the inhibitory effect of SI is reversible (Sarker et al., 1988). Ikeda et al. (1997) have recently shown that an aquaporin gene is tightly linked to a modifier gene at the *MOD* locus and that the aqueporin is not expressed in self-compatible plants homozygous for a mutant *mod* allele. These results

indicate that the aqueporin is necessary for the SI response and the authors propose two models for the role of this protein in the SI response. The first suggests that the water channel transfers water from the stigma surface into the cytoplasm of the stigmatic papillar cells so that less water is available for pollen hydration. The second model suggests that the aqueporin is involved in the transport of small molecules which are either secreted via the aqueporin and inhibit germination of self-pollen and pollen tube growth or are required for the same processes but are sequestered away from the cell wall by the channel. In either model, the aqueporin would be predicted to be a downstream target of SRK activity.

Conclusion

Recent work has provided exciting insights into the molecular mechanism of the SI response in *Brassica*. The identification of potential downstream components of the SI response is particularly interesting. There are, however, still a significant number of questions which remain to be answered. Perhaps the most important of these is: What is the nature of the male component? It seems probable that the combination of both biochemical and map-based approaches will provide an answer to this question in the near future.

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Symposium paper

CONTROL OF FLOWERING TIME IN BANANA (*MUSA* SPP.), A TROPICAL MONOCOT, BY GENETIC TRANSFORMATION

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During the last decade genetic and molecular studies have revealed significant details on the regulation of floral transition, a major developmental switch in plants. Homeotic genes that determine the identity of flower meristems and of floral organs as well as genes inducing precocious flower formation via parallel pathways have been identified and isolated. Recently, the interaction between these genes has been intensively studied in *Arabidopsis*. This progress opens up opportunities for a range of applications in crop plants.

Based on the following characteristics banana appears to be a suitable target for applied research on flowering time control: (i) it is the most important fruit crop in the world and a further yield increase is required; (ii) its generation time may be as long as two years, the shortening of which could be an alternative way of improving crop yield; (iii) it is a tropical and probably day-neutral species flowering throughout the year, and (iv) it has a relatively small genome size and protocols for efficient genetic transformation are available.

Our initial work is focused on the constitutive expression of heterologous 'flowering-time' and homeotic genes in banana. For this purpose, the *Arabidopsis* genes *CONSTANS*, *LEAFY* and *SPL3* fused to different viral promoters are now transformed into banana alone or in combinations. In the future, banana orthologues and homologues of some of these genes will be isolated.

Key words: banana, flowering time, genetic transformation, homeotic gene, *Musa* sp.

Introduction

Developmental characterisation as well as genetic and molecular analyses of reproductive biology in higher plants have become a major focus of plant research in the past decade. Numerous dicotyledonous (including *Antirrhinum majus*, *Arabidopsis thaliana*, *Brassica* spp., *Daucus carota*, *Gasteria verrucosa*, *Impatiens balsamina*, *Nicotiana tabacum* and *Petunia hybrida*) and monocotyledonous (mainly cereals such as barley, maize and wheat) species have been studied in detail facilitated by their well-known floral organ anatomy and/or by accumulated knowledge on their genetics.

However, the majority of the world's population lives in the Third World and depends on endemic tropical crops, some of them using peculiar reproductive strategies. For instance, the flowers of many cocoa cultivars have been reported to exhibit both self- and cross-incompatibility that are expressed only after fertilisation (Cope, 1962). Another interesting example is avocado flowers (Bergh, 1969) that can exclude self-pollination by protogynous, diurnally synchronous dichogamy, i.e.

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the flowers open at two distant periods of the day, the flowers open at two distant periods of the day, first functioning as a female and later as a male. Obviously, there is a need for much more systematic and high-quality research in most tropical crops, especially the tropical food crops.

In order to study the generative phase, plants need to have completed a significant part of their life cycle, which may take several years in many of these perennial tropical species. For example, coconut inflorescences are initiated about three years before their opening and pollen dispersal (Ohler, 1984). In general, the induction of floral organs in angiosperms coincides with the transformation of the shoot apical and lateral meristems into inflorescence and flower meristems. This transition process is under a complex environmental and genetic control, the details of which have begun to be unravelled in recent years (Ma, 1998). Understanding the regulation of flower induction and the control of flowering time in tropical crops could thus result in the production of plants with altered flower morphology, precocious flowering and other potential benefits. Such plants could facilitate further research on their reproductive biology, and accelerate breeding and the characterisation of mutants. More practically, this research could result in increased harvest per time unit due to a shorter generation time, provided the yield potential remains unaffected.

In this paper, the genetic regulation of flower development and of flowering time is briefly reviewed. Then, generative biology and potential applications to banana, the most important fruit crop on earth, are discussed.

Flower development and flowering time control

Floral transition proceeds in two major steps. In the first step, which is called the floral initiation process (FLIP), the shoot apical meristem produces a collection of undifferentiated stem cells: the inflorescence meristem and flower meristems. During FLIP a set of early-acting genes, the flower-meristem-identity genes, are activated, which determine and maintain the floral fate of the meristem. Some of these genes remain active during the second phase when the specific fate of floral organ primordia is established by another set of late-expressing genes, the floral-organ-identity genes.

Flowers, similarly to the segmented body of insects, are built up of repeated, anatomically related structural units, i.e. they are composed of four concentrically arranged rings or whorls: leaf-like sepals in the outermost whorl 1, frequently colourful petals in whorl 2, and stamens or carpels (the respective male or female reproductive organs) in whorls 3 and 4, respectively. The specific identity of these floral organs is regulated by a group of development control (homeotic) genes. Mutations in these homeotic genes result in the formation of normal organs at inappropriate positions, e.g. the conversion of petals to sepals or of stamens to carpels. The expression of these organ-identity genes is largely confined to the specific regions of floral organs and to the primordia where their activity is required.

The systematic investigation of natural homeotic transformations and of transgenic plants ectopically expressing homeotic genes revealed and confirmed the genetic rules that direct flower construction and led to an ingenious concept,

the ABC model (Coen and Meyerowitz, 1991). According to this model, combinations of three classes of homeotic genes are responsible for the generation of the four floral organs described above. Class A genes act in the sepal whorl while they are expressed together with class B genes in the petal whorl. Class B and C genes together encode the production of the stamen whorl and class C genes alone are required for the formation of the carpel whorl. Finally, it has been shown that class A and class C genes mutually inhibit one another, which results in their expression in the first two whorls and the central two whorls, respectively. The predictions of the ABC model have been verified so far in two model plants, *Arabidopsis thaliana* and *Antirrhinum majus*, where the major orthologous homeotic genes have been identified and isolated (Table 1).

Table 1
Orthologous homeotic genes controlling flower meristem identity and floral organ identity in *Arabidopsis thaliana* and *Antirrhinum majus*

Flower-meristem-identity genes		Floral-organ-identity genes	
Arabidopsis	*Antirrhinum*	*Arabidopsis*	*Antirrhinum*
LEAFY (LFY)	*FLORICAULA (FLO)*	Class A:	
APETALA1 (AP1)	*SQUAMOSA (SQUA)*	*APETALA1 (AP1)*	*SQUAMOSA (SQUA)*
APETALA2 (AP2)	*?*	*APETALA2 (AP2)*	*?*
CAULIFLOWER (CAL)	*?*	Class B:	
UNUSUAL FLORAL ORGANS (UFO)	*FIMBRIATA (FIM)*	*APETALA3 (AP3)*	*DEFICIENSA (DEFA)*
FUSED FLORAL ORGANS (FFO)	*?*	*PISTILLATA (PI)*	*GLOBOSA (GLO)*
TERMINAL FLOWER1 (TFL1)	*CENTRORADIALIS (CEN)*	Class C:	
TERMINAL FLOWER2 (TFL2)	*?*	*AGAMOUS (AG)*	*PLENA (PLE)*

The floral homeotic genes are now known to encode putative transcription factors. It is therefore not surprising that the majority of these gene products contain a conserved DNA-binding motif of 56 amino acids, the MADS-box, at their amino-terminal region (reviewed by Shore and Sharrocks, 1995). Many MADS-domain proteins bind to a consensus DNA sequence as homodimers while other members interact with each other by heterodimerisation (Huang et al., 1996). Such MADS-box genes appear to be widespread in angiosperms and even in gymnosperms (Table 2) and may be additionally involved in the sex determination of higher plants (Lebel-Hardenack and Grant, 1997).

These findings and the fact that the interactions of homeotic genes is basically similar in at least two genetically and systematically distant species indicate that the mechanism of flower development in most higher plants may be ruled by common general principles.

Similarly to homeotic mutants, a large number of late-flowering (LF) mutants have been characterised in various plants, most often in *Arabidopsis*. Genetic and physiological analyses of these mutants (Koornneef et al., 1991) revealed the redundant action of the respective genes through parallel pathways (Table 3).

Table 2
Orthologous and homologous floral homeotic genes in diverse plant species

Arabidopsis/Antirrhinum	Other species
LFY/FLO	cauliflower (homologue), tobacco (*NFL*), pea (*PEAFLO*), *Impatiens balsamina* (homologue), rice (*RFL*), *Pinus radiata* (*NLY*)
TFL1/CEN	tomato (*SP*)
UFO/FIM	*I. balsamina* (*IMP-FIM*)
homeotic MADS-box genes:	
AP1/SQUA	cauliflower (*BOIAP1*), *Sinapis alba* (*SAMADSC*), *Silene latifolia* (*SLM4/SLM5*), *I. balsamina* (homologue), maize (*ZAP1*), *Eucalyptus*(*EAP1/EAP2*)
CAL	cauliflower (*BOCAL*)
AP3/DEFA	tobacco (*NTDEF*), *S. latifolia* (*SLM3*), *Rumex acetosa* (*RAD1/RAD2*), petunia (*PMADS1*), potato (*STDEF*)
PI/GLO	tobacco (*NTGLO*), *S. latifolia* (*SLM2*), petunia (*PMADS2*), rice (*OSMADS2/OSMADS4*)
AG/PLE	tobacco (*NAG1*), tomato (*TAG1*), *Brassica* sp. (*BAG1*), *S. latifolia* (*SLM1*), *R. acetosa* (*RAP1*), cucumber (*CUM1/CUM10*), petunia (*PMADS3*), rice (*OSMADS3*), maize (*ZAG1/ZAG2/ZMM1/ZMM2*), ginseng (*GAG2*)
AGL1-AGL17 (*AG*-like)	*Picea abies* (*DAL1*), *P. radiata* (*PRMADS1-PRMADS3*), petunia (*PAGL1*), rice (*OSMADS1*), sorghum (*SBMADS*), maize (*ZAG3-ZAG5*), *Dianthus* sp. (*CMB1*)
other MADS-box genes:	*P. abies* (*DAL2/DAL3*), tomato (*TM3-TM8*), *S. alba* (*SAMADSA/SAMADSB*)

Based on the reaction of LF mutants to environmental signals and on the phenotype of double mutants, the corresponding wild type genes can be divided into at least two major groups (reviewed by Weigel, 1995). A first group of genes is proposed to act via an autonomous (constitutive) pathway that is independent of environmental conditions. A second group of genes probably act through a pathway that specifically responds to inductive photoperiods. Cold temperature can also induce flowering (vernalisation); however, this may not be relevant when working with tropical crops. Finally, a third pathway involves the growth regulator gibberellic acid (GA) as demonstrated recently by Blázquez et al. (1998), who found that gibberellin-deficiency correlated with reduced *LFY* promoter activity and exogenous GA increased *LFY* activity (Blázquez et al., 1997).

Seven 'flowering-time' genes have been cloned up to now (reviewed by Pińeiro and Coupland, 1998): *LD* and *FCA* of the autonomous pathway, *CO*, *FHA*, *FT* and *LHY* of the photoperiodic pathway, and *FPF* of the GA pathway. The encoded protein structures predict widely different functions in accordance with the observed pleiotropic effects of some of these genes. For instance, the FHA protein is probably a blue-light receptor and closely related to the cryptochrome CRY2 that acts antagonistically to phytochrome B (Guo et al., 1998). It is interesting that *LD* and *CO* encode putative transcription factors (Lee et al., 1994; Putterill et al., 1995) of different classes and *FCA* encodes a protein with RNA-binding domains, indicating that it may be a post-transcriptional regulator (Macknight et al., 1997).

Table 3
Genes known to accelerate flowering via parallel pathways

Group/Pathway	Gene	Reference
'Flowering-time' genes		
autonomous pathway	*LUMINIDEPENDENS* (*LD*)	Lee et al. (1994)
	FCA	Macknight et al. (1997)
	FPA, *FVE*, *FY*	Martínez-Zapater et al. (1994)
photoperiodic pathway	*CONSTANS* (*CO*)	Putterill et al. (1995)
	FD, *FE*, *FHA* (=*CRY2*), *FT*, *FWA*	Martínez-Zapater et al. (1994)
	GIGANTEA (*GI*)	Araki and Komeda (1993)
	CARBOHYDRATE ACCUMULATION (*CAM1*)	Eimert et al. (1995)
	LATE ELONGATED HYPOCOTYL (*LHY*)	Coupland (1997)
	Ppd-H1	Laurie et al. (1995)
gibberellic acid pathway	*GIBBERELLIC ACID DEFICIENT* (*GA1-3*)	Blázquez et al. (1998)
	GIBBERELLIC ACID INSENSITIVE (*GAI*)	Wilson et al. (1992)
	FLOWERING PROMOTING FACTOR (*FPF*)	Kania et al. (1997)
Homeotic genes	*LEAFY* (*LFY*)	Weigel and Nilsson (1995)
	APETALA1 (*AP1*)	Mandel and Yanofsky (1995)
	AGAMOUS (*AG*)	Mizukami and Ma (1997)
	OSMADS1	Chung et al. (1994)
	SPL3 (*SQUA* promoter binding protein-like)	Cardon et al. (1997)
Photoreceptors	*PHYTOCHROME A* (*PHYA*)	Bagnall et al. (1995)

Some of these genes have also been expressed in transgenic plants under the control of the constitutive CaMV 35S promoter. The ectopic expression of 35S::*FCA* (Macknight et al., 1997) or 35S::*CO* (Putterill et al., 1995) in *Arabidopsis* resulted in slightly or very early flowering phenotypes, respectively. These experiments proved that the genes identified using LF mutants indeed play a promotive role in flowering. In addition, these transgenic plants also provide a useful tool to determine the sequence and hierarchy of gene cascades in each pathway by measuring their flowering time in different LF mutant backgrounds. According to the presently accepted model (Martínez-Zapater et al., 1994), environmental and internal signals are mediated through redundant pathways of the 'flowering-time' genes (Table 3). Member genes in each pathway form a cascade that leads the signal to the meristem-identity genes. At present, the photoperiodic pathway is characterised best where *FHA* may function as a light receptor and then interacts with *LHY* to increase *CO* expression. *CO*, in turn, is known to activate a range of floral homeotic genes (Simon et al., 1996) including *LFY*, *TFL1* and indirectly *AP1* and *AG*. Similar cascades may function in the parallel pathways. In addition, these pathways appear to form a complex network as loss of function in one pathway can be compensated by another. Of course, other signal transduction pathways may

also have a branch or branches that join the floral program system somewhere. Finally, the network of flowering promoting pathways is not isolated – a similar network of repressive genes can function as demonstrated by the existence of early flowering mutants. The isolation of these genes such as *EMF1* and *EMF2* (Sung et al., 1992) should help to clarify their role in the regulation of flowering and their interaction with the promoting genes. In summary, this model proposes to consider floral transition as a developmental default state that is negatively controlled by a floral repressor, perhaps by the *EMF* genes. The floral repressor would then be under both positive and negative control provided by the promotive and repressive gene cascades as described above, and flowering time would be regulated by the balance of these parallel pathways.

Since 'flowering-time' genes are assumed to act upstream of the homeotic genes, they can be bypassed by the constitutive expression of meristem-identity genes. Though this approach eliminates the dependence on the flowering pathways, it is likely to result in significantly altered flower morphology in the transgenic plants. Indeed, in 35S::*LFY* or 35S::*AP1* transgenic *Arabidopsis* plants most or all lateral shoots were transformed into flowers (Weigel and Nilsson, 1995; Mandel and Yanofsky, 1995). Surprisingly, these plants also displayed early flowering, the extent of which correlated with the activity and the copy number of the gene at least for *LFY* (Blázquez et al., 1997), indicating that *LFY* combines the properties of 'flowering-time' and flower-meristem-identity genes. In addition, *LFY* function appears to be conserved in distant species, as 35S::*LFY,* unlike 35S::*AP1,* induced early flowering in tobacco and hybrid aspen, too. Organ-identity genes are also able to induce early flowering (Table 3). For instance, *Arabidopsis* plants transgenic for 35S::*AG* (Mizukami and Ma, 1997) or doubly transgenic for the homeotic genes *AP3* and *PI* (cit. Nilsson and Weigel, 1997) showed precocious flowering.

Banana: an exotic target for flowering time control

From the viewpoint of a botanist, the banana plant seems to be full of contradictions. It looks like a tree but is a giant herb. It is monocarpic yet perennial, due to vegetative growth from lateral buds of the underground true stem, the so-called corm, which is a rhizome with very short internodes. Located on top of the corm near ground level, the shoot apical meristem initiates the production of 30-40 spirally arranged foliage leaves whose average surface ranges between 1.5 and 3.0 m^2 per leaf. These leaves appear at weekly intervals and their long enclasping sheaths form the non-woody pseudostem. In spite of its 2-6 m height and enormous leaf surface (with a leaf area index of 4 to 6 for fully developed individuals), the plant's root system is surprisingly shallow, only exceptionally reaching a depth of 1 m. However, the total length of the entirely adventitious and extremely dense root system can be as long as 40 km (Swennen and Rosales, 1994).

During floral transition, the compact underground stem quickly elongates with large internodes to an aerial stem or inflorescence stem whose function is

purely connective. The aerial stem grows upwards through the narrow channel in the pseudostem at an average speed of 10–17 cm per day (Devos, 1984). Hence, the aerial stem is weak and has to rely on the support of the pseudostem. The tracheids inside the aerial stem are usually 4 to 8 cm long and are among the longest plant cells known (Skutch, 1932).

The structure and the development of the floral organs in banana can be similarly surprising. While the slow-developing lateral vegetative buds are leaf-opposed, i.e. opposite to the typical axillary position, flower clusters, on the contrary, develop very early in the axils of bract primordia. The floral organs arise in the following order: perianth, stamens and carpels. The perianth (P 1.[(3)+(2)+1]) is strongly zygomorph and is composed of a large compound perigonium of five fused petals and of an adaxial free petal. The androecium (A(3)+(3-1)) consists of the basic two rings of three stamens reduced by one of the inner stamens opposite the free petal. Finally, in the tricarpellary gynoecium (G[(3)]) the three styles are fused and bear a six-lobed stigma. The inferior ovary is trilocular in structure with axile central placentae filled in total with about 300 ovules that are arranged in irregular rows (Stover and Simmonds, 1987).

The flowers are structurally complete or bisexual but functionally they exhibit a sex gradient along the inflorescence axis (peduncle or rachis). The basal 10–15 flower clusters are female (pistillate) with reduced, non-functional staminodes and develop the berry-type, sweet to acid-tasting and usually parthenocarpic fruits. Next, a variable number of neutral flower clusters are formed that can be regarded as hermaphroditic but which never produce fruits. Terminally, 100-300 clusters of male (staminate) flowers can be found in a compact male bud that may be indeterminate or determinate in growth. In most domesticated and triploid bananas the male flowers are completely sterile. Initially, no morphological differences can be observed between flower primordia, indicating that the flowers are sexually bipotent. Indeed, a few wild bananas have preserved the true bisexual character of their flowers. Sex differentiation becomes apparent only when the young inflorescence is approximately 20 cm long and is about half-way up the pseudostem.

The inflorescence of banana is a complex spike where flower clusters developing on protruding glomerules of the peduncle are covered by a single common bract leaf that may peel up after flower opening. Approximately 12 to 20 flowers are clustered uniseriately or biseriately and the total number of clusters can be as high as 300. Many banana cultivars have degenerated inflorescences where either the male bud alone or both the neutral part and the male bud are absent or gradually degenerate during maturation.

It is not known what triggers the floral transition in banana. It is not likely to be regulated by photoperiodism as bananas produce flowers in the tropics as well as in the subtropics throughout the year. Thus, banana can be generally considered as a day-neutral plant, though the related species of *Heliconia* are reported to behave as short-day plants (Criley and Sakai, 1997). Similarly, the total number of foliage leaves may not be the critical factor, unlike in tobacco

(McDaniel et al., 1996), because this parameter can be significantly different in the same cultivar depending on the health and nutrient status of the plant. Therefore, it is possible that a certain leaf area or plant size has to be reached before flower induction takes place in banana.

The duration of vegetative development in banana may be between 180 and 480 days while the generative phase may range from 90 to 250 days. Thus, in total, the time between planting and harvest can be as long as 9 months to more than 2 years. Due to this relatively long life cycle and the high degree of sterility of edible landraces conventional banana breeding has been extremely slow. In addition, the primary objective of classical breeding has been the production of parental lines that are disease- and pest-resistant. Other characteristics such as earliness or short stature have hardly been considered.

Apart from the long life cycle, what is the rationale for investigating flowering time in banana? It is not generally recognised that besides the popular dessert bananas whose international market value exceeds 5 billion US $, 90% of total production worldwide, i.e. 75 million metric tons, are locally consumed by at least 400 million people in less-developed countries after cooking, boiling, baking, frying or even brewing. These figures rank banana as the most important fruit crop on earth. In fact, banana is considered to be the fourth food source in the less-developed part of the world after rice, milk and wheat. For millions in Sub-Saharan Africa, banana is the source of more than 80% of daily carbohydrate consumption. Food is a strategic weapon in these regions of political instability; therefore, an increase in the food supply and in production is of vital importance. For the reasons mentioned above, classical breeding needs other tools in order to achieve an increased banana yield in a relatively short term. The recent progress in developing efficient transformation procedures for banana (Sági et al., 1995) should be useful in this respect. Particle bombardment of embryogenic cell suspensions routinely produces 1 to 3 adult transgenic plants per bombardment. In addition, the available wild diploid species have a relatively small genome (approximately 600 Mb/haploid; Doležel et al., 1994) which could facilitate analyses of the genome.

Therefore, we have started to investigate the expression and functioning of heterologous 'flowering-time' and homeotic genes in banana. For this purpose, chimaeric gene constructs of the *Arabidopsis* genes *CO*, *LFY* and *SPL3* are currently being transformed into a late-flowering landrace. These genes are fused to the 35S promoter as well as to a badnavirus promoter that is highly active in diverse monocotyledonous species. *CO* and *LFY* were selected as the best-known representatives of 'flowering-time' and flower-meristem-identity genes which had already been used with success. As an alternative class, *SPL3*, a putative regulatory gene of *AP1*, was selected because it may interact with other homeotic genes and its expression in *Arabidopsis* had caused no major changes in floral morphology (Cardon et al., 1997). To study possible interactions double and triple transgenics for these genes are being generated. For the ectopic expression of homologous genes in the future, orthologues of some of these genes will also be isolated.

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CURRENT STATUS OF TRANSFORMATION IN BREAD AND DURUM WHEATS AND MODIFICATIONS OF GLUTEN QUALITY*

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The current status of wheat transformation is reviewed in this paper, with the emphasis on biolistic-mediated DNA delivery. The conditions for target explant culture and transformation have been optimised and successfully adapted to a wide range of wheat genotypes, including models and a number of elite varieties. The robust wheat transformation systems developed have now been applied to manipulate the amount and composition of the HMW glutenin subunits which are the major determinants of wheat dough elasticity. SDS-PAGE analysis of seed proteins has indicated that the novel protein bands are expressed in transgenic plants. A 2g Mixograph has been used to analyse the functional properties of additional HMW subunits 1Ax1 and 1Dx5, the results showing that the mixing time and peak resistance are increased, indicating increased dough strength of bread and durum wheats. The alteration of dough strength demonstrates the great potential for wheat quality improvement by genetic modification technologies. Transgenic lines of genetically modified bread and pasta wheats are now being analysed in the field. The stability and heritability of transgenes, both markers (*uidA*, *bar* and *neo*) and genes of interest (1Ax1, 1Dx5), integrated into transgenic bread and durum wheat genomes have been studied and typical Mendelian transmission patterns have been observed in the progenies of T_1, T_2 and T_3 generations of transgenic lines.

Key words: *T. aestivum*, *T. durum*, transformation technology, HMW subunit genes, stability and heritability of transgenes, functional properties, breadmaking quality, quality improvement

Introduction

The application of new technologies to bread and durum wheats based on the identification of agronomically important genes and their expression in transgenic plants has considerable potential for crop improvement. The unique properties of wheat flours and doughs, which allow processing into a range of foods (bread, cakes, biscuits, pasta and noodles) and its wide geographical range (FAO, 1997; Shewry et al., 1997), mean that wheat is one of the three most

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important crops in the world, the others being maize and rice. The total production was about 585 million tonnes of wheat in 1996 compared with 577 million tonnes of maize and about 562 million tonnes of rice (FAO, 1997). Traditional plant breeding has brought about enormous increases in wheat production by the exploitation of genetic variation which exists in the germplasm of wheat or in closely-related species which are sexually compatible, by crossing followed by selection procedures (Bedő, et al., 1998a; Bruns and Peterson, 1998; McIntosh, 1998; Verma et al., 1998). However, wheat improvement by hybridisation is limited due to the restricted gene pool. Advances in plant biotechnology mean that genes can now be used from species which are not sexually compatible with wheat. The introduction of such genes to produce genetically modified (GM) crops, after due consideration and evaluation of possible biological and environmental risks, will greatly assist in improving the quality of wheat and reducing the enormous yield losses attributed to weeds, pests and pathogens, and may also contribute to increased yields (Blechl et al., 1995; Lazzeri et al., 1997; Vasil, 1998).

Compared to some other crop species, including cereals such as maize and rice, the progress of wheat transformation has been slow. Transformation of tomato, potato, oilseed rape, rice and maize has been possible for about ten years and the first transgenic varieties have now reached the market. However, significant progress in wheat transformation has only been made during the past five years, since the initial success achieved in 1992 when the first transgenic wheat plants were obtained (Vasil et al., 1992). Subsequent publications concerning bread and durum wheat transformation have focused on increasing the frequency of transformation by choosing suitable target explants, optimising the culture conditions for these explants, improving the transformation conditions and developing efficient selection systems (Weeks et al., 1993; Becker et al., 1994; Nehra et al., 1994; Zhou et al., 1995; Altpeter et al., 1996; Takumi and Shimada, 1996; Bommineni et al., 1997; Barcelo et al., 1998; He et al., 1998a; Sparks et al., 1998).

These techniques now have great potential for fundamental and applied studies of wheat. In order to modify agronomic traits, it is essential to understand the molecular basis of the characteristics and isolate the relevant genes (Lazzeri et al., 1997; Vasil, 1998). Recently, several interesting characteristics of bread and durum wheat have been modified by genetic engineering, including bread- and pasta-making quality (Blechl and Anderson, 1996; Altpeter et al., 1996; Barro et al., 1997; Blechl et al, 1998; He et al., 1998a; He et al., 1998), functional studies of the *ver* gene (vernalisation) (Chong et al., 1998), the development of a nuclear male sterile hybrid system by the expression of the *barnase* gene (De Block et al., 1997) and resistance to herbicides (Vasil et al., 1992), viruses (Hansen et al., 1995; Karunaratne et al., 1996) and fungal pathogens (Leckband and Lörz, 1998; Romero et al., 1998). The stability and heritability of transgenes in the progenies of transgenic lines have also been studied (Srivastava et al.; 1996, Cannell et al., 1998; Barro et al., 1998).

Particle bombardment has proved to be one of the most powerful methods for wheat transformation (Weeks et al., 1993; Becker et al., 1994; Nehra et al., 1994; Barcelo and Lazzeri, 1995; Altpeter et al., 1996), although several alternative methods have been used (Lazzeri and Shewry, 1993 and Barcelo et al., 1998). He and Lazzeri (1998) described optimised conditions of cell electroporation for wheat transformation and obtained the first fertile transgenic plants of the *Triticeae* by this method (He et al., 1998), using inflorescences of *Tritordeum* (*Hordeum chilense* Roem. × *Triticum turgidum* L. conv. *durum*, Martin and Sanchez-Monge, 1982). Serik et al. (1996) reported successful gene delivery into wheat mature embryos by silicon carbide fibre-mediated DNA transformation. Cheng et al. (1997) most recently described the first wheat transgenic plants obtained by *Agrobacterium tumefaciens* transformation, which is a promising method requiring less equipment and giving low copies of transgene integration. There are also some publications reporting transient expression using this method (Guo et al., 1998; McCormac et al., 1998; Sparks et al., 1998). Chong et al. (1998) described transgenic wheat obtained by the pollen tube pathway, which is a very attractive method but one which many groups have tried to use for cereal transformation with little success.

In this paper, wheat transformation technologies are discussed, including the conditions for improvement of target explant culture and transformation. The transmission pattern of the transgenes in progenies of transgenic plants of bread and durum wheats and *Tritordeum* are analysed and the progress of improving wheat quality is assessed.

Wheat transformation technology

Successful wheat transformation is dependent on eight key factors. The first is an efficient plant regeneration system which is required for the target material as the basis for a high transformation frequency. The second is optimised transformation conditions which reduce the damage to the targeted explants during the transformation process. The third is a suitable selection system which gives a clear discrimination between transformed and non-transformed cells but has little influence on regeneration from transformed tissues or on the morphology, physiology and fertility of transgenic plants. The fourth is molecular confirmation of transgenic plants. The fifth is expression of the transgene. The sixth is the stability and heritability of transgenes in progenies of transgenic plants. The seventh is the correct functional properties of transgene expression products and the eighth is an efficient system allowing the rapid production of homozygous transgenic lines. The first four and the last points are discussed in this section and the other three are discussed in the following sections.

Improvement of tissue culture conditions

Factors which have been found to have a great effect on the plant regeneration and therefore the transformation frequency of bread and durum wheats are the physiological state of the donor plants, explant source, developmental stage of the explant, culture conditions, medium composition and genotype.

The physiological state of the donor plants can be improved partly by the use of artificially controlled environments and the genotype problem can be solved or partly solved by optimisation of culture conditions and culture medium composition.

Immature scutella have proved to be the best target source for bread wheat for *in vitro* culture, though some bread wheat varieties gave good regeneration frequencies from inflorescence tissues (Rasco-Gaunt and Barcelo, 1998). Immature inflorescences are better for *Tritordeum* (a cross between durum wheat and barley) (Barcelo et al., 1994; Barcelo and Lazzeri, 1995; He et al., 1998) and both immature scutella and inflorescence explants may be used for durum wheat, in terms of high frequencies of embryogenic callus induction and plant regeneration (He et al., 1998a; He et al., 1998). The developmental stage of the explant is important for efficient plant regeneration. A suitable stage can be indicated by the morphology of the donor plants and careful checking of the target tissues before isolation.

The list of donor plants used in our laboratory includes twenty bread wheat (*Triticum aestivum* L.) varieties: Avans, Axona, Baldus, Brigadier, Cadenza, Canon, Consort, Florida (model variety with high frequency of plant regeneration in *in vitro* culture), Hartog, Hereward, Hussar, Imp, L88-6, L88-31, Mercia, Pavon, Rialto, Riband, Soisson and Spark, three *Tritordeum* lines: HT28, HT31 and HT174, and four durum wheat (*Triticum turgidum* L. conv. *durum*) varieties: L35, Svevo, Latino × Lira and Ofanto. The list of wheat donor plants is continuously expanding in our laboratory and also in some other laboratories doing similar research.

Apart from explant source and the developmental stage of the explant, exogenous hormone supply has been found to be critical for wheat plant regeneration, especially from the period between induction and the early stages of regeneration. The most effective auxins used for wheat tissue culture are 2,4-D, picloram and zeatin, although some other hormones are also reported in the literature. In general, picloram is better than 2,4-D for embryogenic callus induction and subsequent plant regeneration from the target tissue. A low concentration of picloram is better for scutellum cultures while a higher concentration is necessary for inflorescence cultures; 2,4-D at even lower concentration also gives good results from scutellum cultures. A combination of hormones is normally used during the regeneration period, because different hormones are required to enhance the differentiation of different organs (root and shoot) from cultures. A suitable balance of hormones greatly improves shooting and rooting from cultures; a high concentration of zeatin and a low level of 2,4-D is recommended for high frequency plant regeneration from *Triticeae* species *in vitro*. Generally speaking, one passage on hormone-containing medium is effective for plant regeneration, but in some cases two passages improve the regeneration conditions, especially for poorly-responding wheat genotypes. Recently Rasco-Gaunt et al. (1998) have observed that high concentrations of sugar and a supplement of silver nitrate increase the plant regeneration frequency of bread wheat, suggesting that they protect the explants from the damage caused by the transformation procedure.

Optimisation of transformation conditions

Transformation frequency depends on the above-discussed target regeneration system and transformation conditions. Transformation conditions include methods of transformation, pre/post culture time and the selection agent and its concentration. In addition, the DNA construct, its promoter, intron and concentration all play an important role in obtaining high frequency transformation. For efficient transformation of wheat, three classes of genes are usually used: scorable markers, selectable markers and the gene of interest. Occasionally two classes are involved: a selectable marker and the gene of interest. A single class would be used for transformation in the case of resistance gene transformation which involves only a selectable marker or in the case of transient gene expression experiments which involve only a scorable marker. The scorable marker gene gives an indication of the success of transformation at an early stage (after two days of transformation). The selectable marker allows identification of transformants in a large population of non-transformants by allowing transformants to grow under selection conditions (Lazzeri and Shewry, 1993). The most commonly used scorable and selectable genes are *uidA*, and *bar* and *neo*, respectively. The gene product of *uidA* allows the visualisation of transgene expression *in situ* and gene products of *bar* and *neo* confer resistance to L-phosphinothricin (PPT) and bialaphos or to geneticin sulphate (G418) and paromomycin, respectively. Anthocyanin, luciferase and green fluorescent protein (GFP) genes have also been successfully used as scorable markers in wheat transformation.

Although the effect of the promoter on the stable transformation of wheat does not have a clear picture yet, the significant differences observed in levels of expression between cereal species (such as wheat, rice and maize) and in transient gene expression in wheat and *Tritordeum* transformation (authors' unpublished data) implies that the careful choice of a strong promoter is essential (Lazzeri and Shewry, 1993). The most widely used promoters for wheat transformation are maize ubiquitin and intron, rice actin1-D or CaMV 35S to drive either scorable or selectable genes. Wheat endosperm-specific promoters have been used to control HMW subunit 1Ax1, 1Dx5 and its mutant genes, other genes of interest such as the γ-zein gene (coding for zein proteins involved in protein body initiation and related to "feeding quality" for ruminant animals), and also the scorable marker *uidA*. Typical plasmid vectors used for wheat transformation are pAct1-D GUS containing *uidA* fused to the rice actin1-D promoter; pDE4 containing the *uidA* gene sequence driven by the CaMV 35S promoter; pLRPT GUS containing the *uidA* gene sequence fused to an endosperm-specific HMW 1Dx5 promoter; pAHC25 containing the *uidA* and *bar* genes, each individually driven by the maize ubiquitin promoter and intron; pDE110 containing the *bar* gene driven by the CaMV 35S promoter; pCal-*neo* containing the *npt* II gene driven by the CaMV 35S promoter and Adh1 intron; pHMW1Ax1 containing the 1Ax1 subunit gene, driven by its own endosperm-specific HMW 1Ax1 promoter; and pHMW1Dx5, pLRPT-Dx5-R576 and pLRPT-Dx5-R853 containing 1Dx5 or 1Dx5 mutant subunit genes, driven by

the endosperm-specific HMW 1Dx5 promoter. All the above-mentioned constructs, delivered into a wide range of bread and durum wheat varieties and *Tritordeum* lines, have produced transgenic plants, indicating that wheat transformation technology is becoming more and more routine.

Two methods of wheat transformation to be discussed in this paper are cell electroporation and particle bombardment, with the emphasis on the latter. Biolistic-mediated DNA delivery has the advantage of routinely obtaining transgenic wheat plants at a relatively high transformation frequency (0.1 – 20%) with a wide range of genotypes (Barcelo et al., 1998). However, particle bombardment requires specialised equipment not available in many laboratories. A newly-developed alternative transformation method is cell electroporation. Cell electroporation functions on the principle that when cells which are in contact with plasmid DNA are subjected to a suitable external electric field, transient pores are formed in the plasma membrane and the plasmid DNA can enter through these pores. A high frequency of transient gene expression has been recorded in wheat scutella and *Tritordeum* inflorescences (up to 90%) and the first transgenic plants were obtained from *Triticeae* species by this method (He and Lazzeri, 1998b; He et al., 1998). A detailed analysis of factors affecting electroporation efficiency has been performed. Factors which have been found to have a significant effect are the voltage and pulse length, the volume of electroporation buffer, the osmoticum of the electroporation buffer and medium, the physical state of the pre-incubation medium and the pre-electroporation incubation time and temperature. The method demonstrates potential for use as an alternative wheat transformation tool, if the transformation frequency can reach a level which allows the production of transgenic plants routinely.

A long list of transgenic bread and durum wheat and *Tritordeum* lines has already been recorded from successful particle bombardment using either immature scutella or inflorescence cultures. The transformation conditions found to have significant effects on the efficient transformation of wheat by this method are bombardment conditions and the subsequent selection procedure. The ideal bombardment conditions require a maximum of particles coated with DNA to be delivered into the target cells but with minimum damage to the tissues. The preculture treatment of the target tissue, the use of fine particles of gold or tungsten, an optimum distance between the stopping plate and the target and relatively low helium pressure help to reach this goal.

The selection procedure is critical for efficient transformation due to the co-existence of transformants and a large number of non-transformants under normal culture conditions; the ideal selection system should make a clear discrimination between them. The most widely used selection agents are L-phosphinothricin (PPT) and bialaphos for the *bar* gene, and geneticin sulphate (G418) and paromomycin for the *neo* gene. However, neither of these selection systems has yet reached the effective level required and significant numbers of non-transgenic plants may escape from selection, making the experiment unmanageable. An approach has been taken to solve this problem by prolonging the selection time and increasing the concentration of selection agent by some

laboratories, but the transgenic plants suffer from morphological, physiological and fertility problems. Very recently several alternative methods have been developed for fast screening and assaying the expression of marker genes in cereal and other crop transformants (De Block et al., 1995; Wang and Waterhouse, 1997; Hänsch et al., 1998; Lonsdale et al., 1998), which provide possibilities to resolve the above-mentioned problems and to reduce labour.

Confirmation of transgenic lines

Putative transgenic plants, from which genomic DNA is isolated from leaf material using a CTAB extraction method, are confirmed by molecular analysis. PCR and Southern blot analyses are carried out for the examination of transgenic plants by determining transgene integration and copy number. Histochemical GUS, NPT II ELISA assay and BASTA application are routinely used to identify the expression of the *uidA*, *neo* and *bar* genes, respectively. SDS-PAGE analysis of seed protein is applied to test the expression of HMW glutenin subunit genes. Confirmed transgenic plants are used for further investigation, studying the stability and heritability of the transgenes in progenies of transgenic plants and purifying transgenic lines containing genes of interest for further analysis.

Production of homozygous transgenic lines

Systems which allow the rapid production of homozygous transgenic wheat lines are of interest for the production of GM bread and durum wheat plants with interesting traits. The dihaploid technique has potential to meet such needs, as the technique has been highly developed using anther culture (Chu et al., 1990; Barnabás et al., 1991; He et al., 1993; Szakács and Barnabás, 1995). In addition, there will be great advantage in combining advances from both traditional plant breeding and plant biotechnology (Bedő et al., 1998b; Loerz et al., 1998; McIntosh, 1998), which allow full use of the new techniques.

Stability and heritability of progeny

There is limited published data available on the stability and heritability of transgenic wheat plants (Srivastava et al., 1996; Barro et al., 1997; Cannell et al., 1998). The main reason for this is that successful wheat transformation is only a recent event and the techniques required to approach such a topic are labour- intensive and time-consuming. That transgenes are stable and heritable in the progeny of transgenic plants is a decisive indication of the potential for the successful application of transformation technology in the breeding programme of wheat and other plant species. Although the factors which affect the stability and heritability of transgenes are not yet clear, some reports in wheat have indicated that transgenes are heritable (Cannell et al., 1997; Barro et al., 1997; He et al., 1998). Although the results from Srivastava et al. (1996) show that GUS expression was observed in one out of six T_2 lines and lost in the others, the results from Cannell et al. (1997) indicate that the expression of *uidA*

has been observed over three generations in the case of bread wheat, although a gradual reduction was observed in the T_2 and T_3 generations. In our laboratory, the scorable marker *uidA* has been delivered into different *Triticeae* species including bread wheat, durum wheat and *Tritordeum*. The stability of this gene in a population of eleven cereal primary transformants (four bread wheat, three durum wheat and four *Tritordeum* lines) has been examined (Cannell et al., 1998a; 1998b). The *uidA* gene was transmitted from T_0 to the T_1 generation, in three out of four bread wheat, two out of four *Tritordeum* and three out of three durum wheat lines with a total percentage of 73%. Information based on the above-described results and other reports in maize (Spencer et al., 1992; Register et al., 1994) and rice (Cooley et al., 1995) suggests that the *uidA* gene may be inherently less stable than other genes.

The behaviour of the selectable marker was very stable with respect to integration, inheritance and expression in wheats and *Tritordeum* transgenic plants. Three out of four bread wheat and three out of three durum wheat lines with the *bar* gene gave Mendelian ratios at a frequency of 86% in the population examined. The selectable gene *neo* has also been studied and the results indicate that the *neo* gene was stable from generation to generation in 80% of the population (four out of five bread wheat and *Tritordeum* lines examined), showing Mendelian transmission ratios. One line did not segregate for the *neo* gene and might be homozygous for the *neo* insertion event. Although the behaviour of marker genes is described above, attention is also being paid to the heritability and stability of the genes of interest. The data show that the HMW subunit 1Ax1 and 1Dx5 genes are stably transmitted into progenies of *Triticum aestivum* and *Triticum durum* with Mendelian segregation ratios (Barro et al., 1997; He et al., 1998). The insertion of markers and genes of interest was generally at low to medium copy numbers (5–7) with the range between 2–15 (Barro et al., 1997; Cannell et al., 1998).

The promoter used also has a clear effect on the regulation of transgene expression. For example, the scorable marker *uidA*, driven by either the maize ubiquitin promoter and intron, the rice actin1-D promoter or the CaMV 35S promoter, is expressed in most tissues (leaves, flower organs, embryos and endosperm) of transgenic plants, whilst when driven by an endosperm-specific HMW 1Dx5 promoter, it is expressed only in the endosperm of seeds and is not detectable in other plant tissues.

Improving wheat quality

The main components of wheat flour are starch (ca. 70–80% dry wt), protein (ca. 10–15%), lipids (ca. 1–2% dry wt) and non-starch polysaccharides. Although the amount of protein is low compared to starch, it is of great importance in determining the functional properties of wheat flour, in particular the gluten proteins which account for about half of the grain nitrogen (Lazzeri and Shewry, 1993; Shewry et al., 1997).

Wheat gluten and HMW subunits

Gluten consists of over 50 individual proteins which are classified into two groups. The gliadins are soluble in aqueous alcohols, while the glutenins are alcohol-insoluble in the native state and consist of low-molecular-weight (LMW) and high-molecular-weight (HMW) subunits. The HMW subunit glutenins are major determinants of the functional properties of wheat dough, especially of dough elasticity which is related to breadmaking quality. The relationship of the HMW subunits to gluten elasticity and breadmaking quality is well established and a total of nine different HMW subunit genes have been isolated (Shewry et al., 1995; 1997). Therefore, transformation with HMW subunit glutenin genes has been used to increase the elasticity of bread and durum wheat doughs.

Improvement of the functional properties of bread and pasta wheats

Two publications (Blechl and Anderson, 1996; Altpeter et al., 1996) initially reported the expression of the HMW glutenin genes in bread wheat but did not determine the effects on the functional properties. Results obtained in our laboratory from transgenic bread (Barro et al., 1997) and durum wheats (He et al., 1998; 1998b) have shown increases in dough elasticity resulting from the addition of HMW subunits 1Ax1 and 1Dx5, with good correlation with the expression levels of the transgenes.

We have transformed two near-isogenic bread wheat lines, L88-31 and L88-6 and four durum wheat lines, L35, Svevo, Latino × Lira and Ofanto. The bread wheat line L88-31 is null for the 1Ax1 and 1Dx5 subunit genes and expresses only subunits 1Bx17 + 1By18, while line L88-6 expresses genes for HMW subunits 1Ax1, 1Bx17, 1By18, 1Dx5 and 1Dy10. In L88-6, transgenic lines expressing additional copies of subunit 1Dx5 have been recovered, giving a four-fold increase in the proportion of this subunit. In the bread wheat line L88-31 and durum wheat lines L35, Svevo, Latino x Lira and Ofanto, the expression of additional 1Ax1, 1Dx5 or 1Ax1 and 1Dx5 subunit transgenes has been demonstrated using SDS-PAGE analysis of seed protein. To determine functional properties, a 2g Mixograph system has been used, showing that dough elasticity was increased, especially in the durum wheat L35 line, where the presence of the additional subunit 1Dx5 resulted in highly elastic dough. These results demonstrate that genetic manipulation can be used to improve a quality trait of bread and durum wheats (Lazzeri et al., 1997; Barro et al., 1998; He et al., 1998). The transgenic bread and durum wheat lines are now being grown in the field in England and in Italy and more information will be gained by large-scale analyses and functionality tests on bulk grain.

We are currently investigating the molecular basis for the functional properties of the HMW subunits by producing transgenic wheat (lines L88-31 and L88-6) expressing subunit 1Dx5 genes which contain different lengths of repeated sequences (D'Ovidio et al., 1997). We have so far recovered transgenic lines with constructs coding for both shorter and longer subunit proteins and confirmed these by PCR. Analysis of the seed proteins of several lines by SDS-PAGE has always shown a corresponding additional protein of the expected *Mr*.

Conclusions

Current progress in wheat transformation

Great progress has been made in wheat transformation during the last five years. Since transgenic wheat plants were first produced in 1992, the technology of particle bombardment transformation has been well developed and a number of important parameters have been analysed and optimised. This implies that the biolistic transformation method will still be the leading method for wheat transformation in the foreseeable future, although a challenge from the main alternative method of *Agrobacterium*-mediated transformation can be expected.

Prospects

The use of transgenic wheats with genetically modified traits such as herbicide resistance or protein composition may have a beneficial effect on the environment by significantly reducing the use of agrochemicals currently used to control weeds and pathogens and by providing higher quality for human beings and livestock. Further, genes for manipulating starch/protein/oil quality and quantity, as well as to confer resistance to biotic and abiotic stresses such as temperature, drought, and salinity/metal toxicity, are also being isolated and studied and will be used to produce transgenic wheat plants in the near future. Finally the manipulation of photosynthetic efficiency and flowering time, and source/sink relationships could be used to increase crop yields.

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Book review

E. BALÁZS, J. DOHY, D. DUDITS, A. FEHÉR, E. GERGÁTZ, B. HARRACH: Biotechnológia: lépéstartás Európával In: Magyarország az ezredfordulón. Stratégiai kutatások a Magyar Tudományos Akadémián II. Az agrárium helyzete és jövõje (Biotechnology: Keeping up with Europe In: Hungary at the millennium. Strategic research at the Hungarian Academy of Sciences. Part II: The present and future of agriculture.) Hungarian Academy of Sciences 1998, pp. 157. ISBN 963 508 064 6

The surge in the development of biotechniques and genetics witnessed in the second half of the 20th century made it clear that the 21st century will be the century of biotechnology. Its economic, social, philosophical and ethical impact can be compared at most to the great geographical discoveries. Since new technologies may play a similarly stimulating role in agriculture and the food industry or in medicine as the car industry does in industry, the necessity for its rapid integration in the economy is vital and evident. This is particularly true of the 'emerging markets', including former controlled economies trying to catch up or keep up with the developed half of the world. In these typically Eastern European countries the private sector of the economy (with the exception of international companies) is still unable and the state sector already not able to be the sole financier of biotechnology research. Moreover, the relatively small markets of these countries force the state to play a more active role. On one hand, there is a tendency among national firms and even research workshops to give up costly R&D and engage merely in production (usually for foreign companies) in order to survive the constraints of decreasing state subsidies.

However, due to their lack of capital, such commission work is usually just an episode, lasting for various lengths of time, in the history of a company before the final chapter: its procurement by a foreign company with solid capital. There is also social pressure, or (as it has no social or economic power) a moral pressure from the public, because of the continuous brain drain and the pauperisation of the scientific community. On the other hand, the unique state of the economy in such countries, which is a state of transition, may also afford an opportunity for change similar to the way in which Japan concentrated its efforts after World War II (with foreign resources) to rebuild and then to launch itself into a leading position in advanced technologies – within a few decades.

Therefore, politicians who are in a position to make decisions and to distribute resources, but not in a position to do this without help and advice, should be informed about the importance and advantages of actively supporting biotechnology and of how to cope with the possible concern expressed by the public on health, moral and environmental issues.

A book has been compiled by János Dohy and Dénes Dudits, both members of the Hungarian Academy of Sciences, dealing with the present and future of plant-, animal- and animal health-related biotechnology in Hungary. Each field is reviewed by leading experts.

The book is divided into three parts. The first part introduces the different fields and strategies of plant biotechnology and gives a general overview of its world-wide application. The measurable economic effects of biotechnology are illustrated with proven examples. The chapters cover general trends in international development, the present situation and future possibilities in Hungarian agriculture. The main national research centres and workshops and their scope of research are highlighted.

In Part Two, animal biotechnology is discussed in detail (e.g. macro- and micromanipulation of the embryo, trans-genesis, cryopreservation, artificial insemination) with special emphasis on the present situation in Hungary. Current trends and possible ways of utilisation are outlined. The integration of biotechnology in to national breeding strategies and the possible 'breakthrough' points are thoroughly discussed as well. The authors also analyse the relation between the state and the research community and the policy on science and financing. Besides criticism, short- and long-term actions are recommended and outlined.

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Part Three is devoted to the use of biotechnology in animal health care. The various chapters review different diagnostic methods (RFLP, RAPD, PCR, ELISA) and biotechnologically produced vaccines (ISCOM). It also discusses undergraduate and postgraduate education, state support and the integration of research and production.

Recently, the Hungarian Parliament has approved a law regulating the release of genetically improved organisms. Thus, Hungary can be a significant partner in the development and application of this new technology based on molecular and cellular biology approaches in agriculture.

B. BARANYAI

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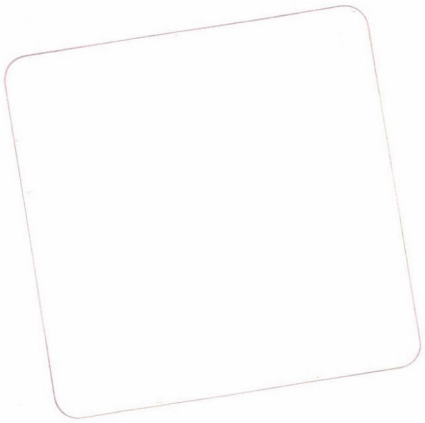

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